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RESEARCH REPORT

STATE-OF-THE-ART STUDY

ON

LEECH REPELLENTS (U)

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AD413979

STATE-OF-THE-ART STUDY

ON

LEECH REPELLENTS (U)

by

Walter H. Veazie, Jr., and Reynolds C. Overbeck

July 10, 1963

For
Project AGILE
Advanced Research Projects Agency

Under
Contract SD-171, ARPA Order 324

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August 2, 1963

Mr. Floyd M. Van Hoosen
Advanced Research Projects Agency
Room 2E163
The Pentagon
Washington 25, D. C.

Dear Mr. Van Hoosen:

Enclosed is our report titled "State-of-the-Art Study on Leech Repellents" (U). This report provides detailed information from selected references, which are acknowledged in Appendix B, because the source material is not readily available. Further, since this is the first state-of-the-art study on leech repellents, it was deemed desirable to consolidate as much pertinent information and data as available into the report.

We would appreciate any comments which you or your associates might care to make with regard to this study.

Very truly yours,

John W. Murdock
ael

John W. Murdock
Project Director
Battelle-RACIC

JWM/mb
Enc.

[redacted]

DEDICATED TO THE ADVANCEMENT OF SCIENCE

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STATE-OF-THE-ART STUDY ON LEECH REPELLENTS (U)

by

Walter H. Veazie, Jr., and Reynolds C. Overbeck

INTRODUCTION AND OBJECTIVE

This two-month study has been conducted with the objective of determining the state of the art, and of learning where gaps exist in the U.S. knowledge of leech repellents. A list of the publications screened in order to collect information for this study is provided in Appendix A. Appendix B provides the acknowledgement of individuals, considered to be authorities on leech repellents and closely related research, who were contacted during the course of this study. The information obtained is applicable to Vietnam and Thailand as well as other tropical lands.

This report summarizes the information currently available on repellents for aquatic and terrestrial leeches. Information is provided that reflects early work on leech repellents, contemporary studies, and current activity. The geographical distribution of leeches is described. Areas which are considered to merit investigation, as well as chemicals which have promise of providing repellency, are identified; in this connection, techniques which have been used to evaluate repellency are discussed briefly. Also, recommendations of research effort that would fill some of the gaps, are presented. The organisms, repellents, and chemicals discussed are of direct medical or veterinary importance.

Many species of aquatic and terrestrial leeches have been recognized since ancient times as organisms which should be avoided. As the U.S. has become more deeply involved in tropical regions of the world, the concern over the availability of effective leech repellents has increased. During World War II the leech was recognized as an undesirable parasite by U.S. troops. More recently, the Peace Corps has made mention of terrestrial leeches in their publication "A Guide to Health", unfortunately, however, no repellent or control method is indicated.

The leech cannot be simply dismissed as an indigenous pest in countries like Thailand or Vietnam. As far as is known, the leech is not a carrier of any disease. However, laboratory studies[1,2*] indicate the possibility of its being a vector of blood-borne viruses.

Boynton[1] found that rinderpest, an infectious disease, could be transmitted to cattle by the water leech Hirundinaria manillensis. On the basis of his investigation, he suggests the possibility that the leech is a mechanical vector of diseases such as leptospirosis.

A laboratory study was conducted by Shope[2] to determine if leeches could serve as a virus vector. Medicinal leeches, Hirudo medicinalis, fed on swine infected with hog cholera virus, contained the virus for as long as 87 days after their blood meal. Eleven leeches, so fed, were placed on uninfected swine to determine if the cholera virus could

* Superscript numbers refer to items listed in the References.

be transmitted. In three instances, the infected leeches transmitted the cholera virus to the swine in the course of normal feeding; the times following the infective meal were 9, 18, and 34 days, respectively. Also, myxoma virus persisted in leeches for as long as 154 days after the ingestion of a blood meal from rabbits infected with myxomatosis. On the basis of his study, Shope concludes that leeches are not biological carriers of either hog cholera virus or myxoma virus, but instead carry these two agents mechanically in their gastrointestinal tracts; by so doing, they appear to protect the viruses from various deleterious chemical and physical influences to which the viruses would be exposed in the open. It is further speculated that leeches could, indeed, play a role in perpetuating blood-borne viruses.

Further, the bite inflicted by the leech results in irritation with associated scratching directed toward allaying the irritation. Thus, a site for a secondary infection occurs and this frequently leads to abscesses and ulcers, which because of their slow healing in hot and humid climates, are extremely serious. The attack of the leech is particularly insidious because the leech may attach itself, make its bite, and be partially engorged with blood before its presence is discovered. This may give its victim a real shock of surprise, especially if he is relatively unfamiliar with the habits of the leech. It is not surprising, therefore, that people are said to react with a sense of panic on finding themselves the host to one or many leeches. The psychological aspect of this surprise, and perhaps panic, is usually significant.

The theoretical criteria for an ideal repellent[3] may be considered to include: (1) complete protection of the treated areas for several hours under all conditions of weather and infestation; (2) protection against all biting insects and leeches; (3) lack of toxicity and irritation to skin and mucous membranes; (4) lack of unpleasant odor; (5) harmlessness to clothing; (6) cosmetic acceptability; (7) ease of application; and (8) low cost. It was found during World War II that the most important criterion of repellents is their cosmetic acceptability. Regardless of how perfectly a repellent meets the above criteria, it is worthless if not used. Appendix C provides data on the cost of chemicals used in leech-repellent formulations.

The British and French Colonials in tropical lands have for the most part accepted leeches as part of the environment. The natives of leech-infested areas are accustomed to them and do not take kindly to protective or control techniques; they simply accept the leech as a pest.

SUMMARY

On the basis of the literature reviewed and discussions with investigators generally considered to be most knowledgeable, it has been determined that natives in areas inhabited by leeches accept them as part of the environment. The major concern about leeches is expressed by personnel who enter such areas and stems from the unfavorable psychological effect which leeches produce. M-1960, diethyl toluamide, and dimethyl phthalate insect repellents will provide for leech repellency if used by personnel along with adequate clothing which provides a mechanical barrier. While these repellents are available and provide a high degree of protection from terrestrial leeches and to a much

lesser extent from aquatic leeches, they lack widespread acceptance owing to the incomplete protection provided, lack of persistence, cosmetic disadvantages, unavailability, and to the fact that in some cases impregnated uniforms are required and these are uncomfortable in tropical regions. Owing to the lack of persistence, there is currently not an acceptable aquatic-leech repellent available.

Gaps in our knowledge of effective leech repellents and leech morphology and taxonomy are identified. A long-range program to fill in these gaps is presented.

LEECH REPELLENTS

Historical Review of Early Repellents

Stammers[4] provides a vivid account of the historical interest in leeches. He reviews the reports prepared by various authors on leech infestation; on the basis of these, it seems that the resulting inconvenience and harm have been considerable. Among the early repellents were lemon juice used by people walking through the jungles and carbolic acid applied around the tops of hunting boots.

Coulter[5] reported that certain natives in Ceylon appear to be immune to leech attacks and, entirely bare footed, can walk among leeches with absolute immunity. Other natives, however, even of the same family, are immediately attacked. Coulter utilized moistened tobacco leaves as a means of preventing leech attachment. He used a pair of specially prepared linen anklets which consisted of two layers of material with an intermediate layer of tobacco; when going out into leech-infested areas he dampened the anklets. With these on his ankles, he was able to walk in patches abounding with leeches, without sustaining any leech attachment.

De Mesa[6], in discussing forest pests in the Philippines, indicates that leech attack may be prevented by wetting one's stockings with a thick solution of soap and nicotine sulphate; this mixture discourages leeches. Further, he indicates that wearing spiral puttees or leggings may also help by providing a mechanical barrier.

Harrison[7] provides a concise review of the leech, its method of attack, and repellents, and points out where information is lacking.

Contemporary Repellent Studies

General

Studies on possible leech repellents have not been the primary mission of the investigators who have reported on repellents. As an example, the studies on terrestrial leech repellents were all of secondary concern to Traub, Audy, Harrison, and Newson; their major concern was scrub typhus and other medical problems in tropical medicine.

The aquatic leeches encountered by Newson and Traub[8] were of the Hirundinaria manillensis species that, because of their large size, are repelled by clothing. Neither of these investigators is aware of any repellent studies on the Limnatis nilotica. The literature indicates that this type of leech prefers to reside in body openings; and seems to seek darkness and the soft epidermis provided by the nasopharynx, urethra, etc., rather than the external skin for attachment and engorgement.

Gavrichenkov[9] conducted a study from 1955 to 1957 of domestic animals infested by Limnatis nilotica. His study, however, was concerned with domestic animals, and his recommendation indicated that primitive watering places used for cattle must be eliminated.

Table I provides a summary of the various repellents studies uncovered, the organisms repelled, and the associated references.

Stammers[4] conducted a laboratory study on Haemadipsa zeylanica from the Burmese-Indian border. The leeches were placed in glass cylinders 8 inches in diameter and 10 inches deep. Each cylinder contained cut turf, moss, and leaves which were kept moistened. The lid of the container consisted of a piece of tightly tied cloth, the threads of which were woven close enough and were sufficiently strong to prevent the leeches from escaping by penetration or severance.

A search was made for a repellent which would prove so distasteful to leeches that when it was applied to skin or clothing the biting would be totally prevented. The test method devised consisted of placing potential repellent solutions on pieces of fine filter paper, each being 2 x 1 inch and receiving 20 cubic millimeters of the repellent solution per square inch. The papers were then laid aside for 3 hours and subsequently tested separately, each being placed in the path of a leech so that the latter could come in contact with it. If the substance under examination was without repellent power, the leech crossed the paper without hesitation; a repellent effect was indicated by a quick withdrawal of the anterior sucker followed by a change of direction, which enabled the paper to be avoided. At the end of 6 hours and also 24 hours, the treated papers were presented again so as to obtain an estimate of the repellent persistency. Those substances which were promising were tested on the skin of man, the same quantity, namely, 20 cubic millimeters, of solution being spread over each square inch of skin. Effectiveness as a repellent was indicated by failure of the leech to cross the treated areas and refusal to bite.

Altogether, 79 substances were investigated on pieces of filter paper. One, namely, hydroxycitronellal, stood out as markedly superior to all of the others; 1 part of hydroxycitronellal per 100 parts of arachis oil was sufficient to repel leeches and none of the other substances was effective at this low concentration. Next in order of effectiveness were five substances: dimethyl phthalate, diethyl phthalate, ethyl hexanediol, vanillin, and nicotine. All these repelled at a concentration of 1 part in 60. Less effective were the following 11 substances which repelled at a concentration of 1 part in 30: citronellal acetate, dimethylhexahydrophthalate, dimethyl-4-tetrahydrophthalate, cis-iso-eugenol, methyl-benzyl maleate, methyl iso-propyl phthalate, oleum cassiae, beta-phenoxy ethyl acetate, phenyl methyl maleate, triethyl citrate, and tri-iso-propyl citrate.

TABLE I. SUMMARY OF INFORMATION ON LEECH-REPELLENTS STUDIES

Name or Composition of Repellent	Leech Repelled or Studied	Extent of Repellency	Reference
M-1960: n-butylacetanilide, 2-butyl 2-butyl-2-ethyl-1,3 propanediol, and benzyl benzoate (equal parts) Tween 80 (10%)	Hirundinaria manillensis Haemadipsa zeylanica Haemadipsa picta	Protection after one washing for land leeches; after six washings ineffective. Against aquatic leeches ineffective.	10, 11, 14, 17
M 1960: n-butylacetanilide, 2-butyl-2-ethyl-1,3 propanediol, and benzyl benzoate (equal parts) Tween 80 (10%)	Haemadipsa	Complete protection for parts of the body covered with impregnated material, owing to vapor tension of M-1960.	16
DMP: Dimethyl phthalate (90%) and Santocel C (10%)	Hirundinaria manillensis Haemadipsa zeylanica Haemadipsa picta	Water leeches little affected by ointments. Land leeches affected for ~1 hour.	10
DMP: Dimethyl phthalate	Haemadipsa sylvestris	A dose of 4.0 cc/sq ft was effective for 6 days under favorable conditions. Strips tested with 2.0 cc/sq ft after 2 hours in running cold water were still 100% effective; after 3 hours, only 60% effective; and after 4 hours, ineffective.	13
Dimethyl phthalate	Hirudo medicinalis related to repellency of Haemadipsa zeylanica	Laboratory study showing fresh applications to be effective	12
Cream: Dimethyl phthalate (25%), white wax (18%), and arachis oil (57%)	Haemadipsa zeylanica	Cream applied to shoes and legs up to the knees effective as a repellent.	4
Dimethyl phthalate, 4 parts Rutgers 612 (2-ethyl-1,3-hexanediol), 3 parts } 85% Dimethyl carbate, 3 parts Santocel C 15%	Hirundinaria manillensis Haemadipsa zeylanica Haemadipsa picta	Water leeches little affected by ointments. Land leeches affected for ~1 hour.	10
Ethyl-beta-phenyl hydracrylate (88%) and Santocel C (12%)	Hirundinaria manillensis Haemadipsa zeylanica Haemadipsa picta	Water leeches little affected by ointments. Land leeches affected for ~1 hour.	10

TABLE I. (Continued)

Name or Composition of Repellent	Leech Repelled or Studied	Extent of Repellency	Reference
Indalone	Hirudo medicinalis related to repellency of Haemadipsa zeylanica	Laboratory study showing fresh applications to be effective.	12
Rutgers 612: 2-ethyl-1,3-hexanediol	Hirudo medicinalis related to repellency of Haemadipsa zeylanica	Laboratory study showing fresh applications to be effective.	12
Rutgers 612: 2-ethyl-1,3-hexanediol	Haemadipsa sylvestris	As effective as DMP, but effect wore off more rapidly, with only 5% effectiveness after 24 hours. Ineffective after one washing, or 1 hour in running cold water.	13
M-2065: Undecylenic acid (29%) n-propylacetanilide (29%) n-butyl-4-cyclohexene-1,2-carboximide (29%) Lindane (3%), and Tween 80 (10%)	Haemadipsa picta Haemadipsa zeylanica subagilis	Effective after one washing; after six washings completely ineffective.	11
M-2066: Undecylenic acid (29.66%) n-propylacetanilide (29.66%) n-butyl-4-cyclohexene-1,2-carboximide (29.66%) Lindane (1.00%), and Tween 80 (10.00%)	Haemadipsa picta Haemadipsa zeylanica subagilis	Effective after one washing; after six washings completely ineffective.	11
DBP: Dibutyl phthalate	Haemadipsa sylvestris	Only 62% effective after immediate application. Completely ineffective after either one washing, 1 hour in the sun, or 1 hour in running cold water.	13
DDT: Dichlorodiphenyltrichlorethane, 5% in kerosene	Haemadipsa sylvestris	No repellent effect.	13

TABLE I. (Continued)

Name or Composition of Repellent	Leech Repelled or Studied	Extent of Expellency	Reference
Coconut oil	Not specified	Malayan natives coat themselves with coconut oil to counter leeches. Oil provides a mechanical disadvantage to the leech sucker.	15
Tobacco	Haemadipsa zeylanica	Linen anklets containing moistened tobacco effective for daily work.	5
2-butyl-2-ethyl-1,3-propanediol, 400 grams in 11 liters of water	Haemadipsa	Complete protection because of vapor tension. Provides slight skin irritation. Not as effective as combined materials in M-1960.	16
Benzyl benzoate, 300 grams in 11 liters of water	Haemadipsa	Complete protection because of vapor tension. Provides slight skin irritation. Not as effective as combined materials in M-1960.	16
n-butylacetanilide, 200 grams in 11 liters of water	Haemadipsa	Complete protection because of vapor tension. Provides slight skin irritation. Not as effective as combined materials in M-1960.	16
Tween 80, 100 grams in 11 liters of water	Haemadipsa	Complete protection because of vapor tension. Provides slight skin irritation. Not as effective as combined materials in M-1960.	16
Hydroxycitronellal	Haemadipsa zeylanica	More effective than the cream of dimethyl phthalate, but more expensive.	4
Diethyl toluamide	Haemadipsa zeylanica Haemadipsa picta	When applied to the skin was extremely repellent. It is highly soluble in water; hence, when skin is washed, compound loses most of its repellency.	18

All of the above, with one or two exceptions, were effective at 3, 6, and 24 hours after application. The exceptions were oleum cassiae and citronellal acetate, both of which failed to repel after 6 hours. Nicotine (probably sulphate), although uncertain in its action, produced marked paralysis after several contacts and was unique in this respect.

The following 62 substances were either completely ineffective or possessed very little power to repel:

> Cis-acetyl-iso-eugenol, amyl acetate, amyl butyrate, amyl salicylate, arachis oil, beeswax, benzene hexachloride, benzyl benzoate, iso-butyl phenyl acetate, n-butyl iso-propyl phthalate, cetyl-trimethyl ammonium bromide, p-chloro-ethyl ether of eugenol, cholesterol, citric acid, citronellal methyl phthalate, coumarin, cyclohexanone, dibutyl phthalate, dichlorodiphenyltrichlorethane, diethyl cinnamate, diethyl adipate, dimethyl maleate, dimethyl thianthrene, diphenyl phthalate, di-iso-propyl adepate, ethyl benzoate, ethylene glycol dioleate, ethyl lactate, ethyl phenyl-acetate, ethyl iso-propylphthalate, fixone, geranyl acetate, geranyl-methyl phthalate, guanidine nitrate, hexyl resorcinol, beta-hydroxy-quinoline, indalone, ionone, lemon juice, linalyl acetate, methyl benzoate, methyl-n-butylphthalate, methyl-cyclohexanyl-acetate, methyl-cyclohexanol, methyl-cyclohexanone, methyl-cyclohexyl methyl phthalate, methyl phenyl-acetate, methyl o-toluate, a-naphthol, oil of white thymi, paraffin (hard and soft), potassium acid phthalate, di-iso-propyl phthalate, pyrethrum, safrole, sodium iso butyrate, sodium citrate, sodium valerate, tributyl phthalate, tri-n-propyl citrate, wood alcohol, and DDT, which is used extensively as an insecticide.

From the viewpoint of protection afforded, hydroxycitronellal was compared with dimethyl phthalate. As both gave such good results it was not easy to make a decision between the two; both were effective provided that no accessible skin was left uncovered. Nevertheless, the impression was gained, as in the laboratory tests, that hydroxycitronellal possessed the greater power to repel and that it would be the substance of choice if the question of supply and cost were not considered. Neither produced any irritant effect on the skin.

Further, hydroxycitronellal, unlike other citronella compounds, was effective over a long time period. Its odor is slight but agreeable, and it has been shown to act as a repellent against mosquitoes. However, since the supply of this compound was limited, the price was high, and there was no certainty that it might not induce a dermatitis in certain individuals living under hot and humid conditions, attention was turned to the four substances which stood next in repellent power, namely, dimethyl phthalate, diethyl phthalate, ethyl hexanediol, and vanillin. Ethyl hexanediol was not found to be a successful repellent against the majority of disease-carrying mosquitoes. Diethyl phthalate and vanillin suffered from this disadvantage to an even greater degree. As dimethyl phthalate is a liquid which easily escapes from containers, creams incorporating this compound were evolved. Cream preparations of dimethyl phthalate that were found to be the most satisfactory were:

(1)	Dimethyl phthalate	25%
	White wax	18%
	Arachis oil	57%
(2)	Dimethyl phthalate	50%
	White wax	10%
	Arachis oil	10%
	Wood alcohol	30%
(3)	Dimethyl phthalate	50%
	Wood alcohol	50%
(4)	Dimethyl phthalate	25%
	Lanette wax	25%
	White wax	15%
	Hard paraffin	15%
	Wool fat	20%

The first preparation was considered the best since it spread well on skin and leather. Moreover, the wax and arachis oil augmented the efficacy of the preparation by delaying evaporation of the active material and thus retained it longer in contact with the surface to which it was applied.

British and American troops operating in the jungles of India and Burma during World War II found that dimethyl phthalate, which was effective for protection against chiggers and mosquitoes, was fairly effective against terrestrial leeches[17]. This compound unfortunately has disadvantages in that it tends to produce sensations of burning when applied directly to the skin, especially on cut and abraded surfaces, and on the more sensitive body portions such as the genito-urinary region and conjunctivae.

Blyth[15], accompanying military patrols in Malaya, noticed that leeches did not lodge on skin areas soaked in dimethyl phthalate. Native porters, utilized with the patrols, used coconut oil smeared on the skin as a protection. This appears to be more of a mechanical disadvantage than a repellent to the sucker of the leech.

M-1960 Repellent Studies

Audy and Harrison[14] conducted field tests of the leech repellency of M-1960 impregnated in uniforms. For the tests, 1 gallon of the solution of the M-1960 concentrate was emulsified in water to a volume sufficient to treat 28 sets of uniforms using hand application, or approximately 13 uniforms using portable laundry equipment.

Uniforms impregnated with M-1960 have a distinctive, although not an overpowering, odor which does not seem to be offensive to the majority of wearers. Under the environmental conditions of test, there were no unpleasant effects on the wearer except that freshly treated uniforms, when worn next to the skin, were likely to cause a slight burning sensation when the subject was perspiring. The greatest disadvantage of the repellent in the clothing was that it softened objects held in the pockets such as plastic watch glasses, fountain pens, and other plastic materials, and made them sticky. For

the tests, standard U.S. tropical light-weight uniforms were impregnated with a dosage of approximately 1.85 cubic centimeters per set of trousers and jacket, underskirt, and socks.

Care was taken in selecting the human-subject volunteers for this study, in an attempt to assure a group homogeneous in every respect but age. The volunteers were divided up into parties, each consisting of as many men as there were repellent treatments under test. A different repellent treatment was applied to each man in any one party. The parties were subjected to field conditions such as clearing out undergrowth, walking through swamps, etc. On this basis, the members of each party were presumed to have had closely comparable exposures.

At the beginning of this study, every leech bite on the volunteers was cauterized with a silver-nitrate stick. But, while this treatment stopped the bleeding, it delayed healing, and was soon abandoned.

Tests were carried out against Hirundinaria manillensis (aquatic), Haemadipsa zeylanica (ground terrestrial), and Haemadipsa picta (bush terrestrial).

An experiment in investigating the repellency of M-1960 toward aquatic leeches (Hirundinaria manillensis) was conducted by having the volunteers walk slowly for a distance of 100 yards through leech-infested swamp which, on the average, was ankle deep in water. The volunteers were then examined; fresh bites and attached leeches were counted as bites, and leeches free on the clothing were counted as "unattached". Eighteen volunteers were utilized, six in shorts with bare legs and feet, and six with treated and six with untreated uniforms consisting of trousers tucked into socks which were protected by rubber sandals. The results of this experiment are summarized in Table II.

TABLE II. EFFECT OF M-1960 REPELLENT TREATMENT ON CLOTHING[14] – AGAINST AQUATIC LEECH (TROUSERS TUCKED IN SOCKS)

	With Treated Clothing		With Untreated Clothing		With Bare Legs	
	Leeches	Bites	Leeches	Bites	Leeches	Bites
	0	0	0	0	0	5
	0	0	1	0	0	0
	0	0	1	0	0	1
	0	0	3	0	0	4
	0	0	3	0	0	2
	0	0	0	0	0	4
Total	0	0	8	0	0	16
Mean	0	0	1.33	0	0	2.67

It is evident from Table II that the clothing provided efficient protection against the aquatic leeches investigated in this experiment. The mechanical barrier thus set up was so effective that little was left for the repellent to do. To provide a more rigorous test, a generally similar experiment was performed with the trousers not tucked in but

hanging loosely at the level of the ankles and the socks rolled at the top, thus allowing the leeches free entrance between the trousers and socks. In these tests, the results of which are given in Table III, the volunteers stood knee deep in leech-infested pools for about 15 minutes.

TABLE III. EFFECT OF M-1960 REPELLENT TREATMENT ON CLOTHING[14] – AGAINST AQUATIC LEECH (TROUSERS NOT TUCKED IN SOCKS)

	With Treated Clothing		With Untreated Clothing		With Bare Legs	
	Leeches	Bites	Leeches	Bites	Leeches	Bites
	0	0	1	7	0	10
	0	0	0	4	0	16
	0	0	0	1	0	5
	0	0	0	0	0	3
	0	3	0	3	0	1
	0	0	1	5	0	1
Total	0	3	2	20	0	36
Mean	0	0.5	0.33	3.33	0	6.0

It is clear from Table III that the treated uniforms offered good protection even when free access was offered to the leeches.

As a further check, the leech repellency of these uniforms after being washed twice with soap and cold water was tested by volunteers who stood for over 1 hour in heavily infested pools. Table IV shows the results.

TABLE IV. EFFECT OF TWO WASHINGS ON M-1960 REPELLENT-TREATED CLOTHING[14] – AGAINST AQUATIC LEECHES (TROUSERS NOT TUCKED IN SOCKS)

	With Treated Clothing		With Untreated Clothing	
	Leeches	Bites	Leeches	Bites
	5	3	9	6
	0	0	26	11
	4	5	41	21
	0	5	33	14
	8	2	5	1
	2	0	22	12
	0	0	5	7
Total	19	15	141	72
Mean	2.71	2.14	20.14	10.29

Under these stringent conditions, the twice-washed treated clothing no longer offered complete protection. However, the advantage over untreated uniforms is clearly so great that were a mere mechanical barrier added such as tucking the trousers in the socks, the protection afforded by twice-washed treated uniforms would be as good as could be achieved.

In the investigation on terrestrial-leech repellency, the volunteers were assigned a task of clearing sections of undergrowth and were recalled to the examination point at half-hour intervals for the purpose of counting the land-dwelling leeches, Haemadipsa zeylanica. Table V reports the results. Once again, the M-1960 treated uniforms provided considerable protection.

TABLE V. EFFECT OF M-1960 REPELLENT TREATMENT ON CLOTHING - AGAINST TERRESTRIAL LEECHES[14]

	With Treated Clothing			With Untreated Clothing			With Bare Legs		
		Unfed			Unfed			Unfed	
	Fed	Alive	Dead	Fed	Alive	Dead	Fed	Alive	Dead
	0	0	7	2	10	0	8	0	0
	0	0	21	3	10	0	11	1	0
	0	0	8	8	27	0	9	3	0
	0	0	0	5	16	0	11	0	0
	0	5	1	1	0	0	8	3	0
	0	1	1	1	3	0	7	0	0
Total	0	6	38	20	66	0	54	7	0
Mean	0	1	6.33	3.33	11.0	0	9.0	1.17	0

It was impractical or invalid to make counts of terrestrial-leech bites as had been done in the aquatic-leech study, summarized above, because the bites were smaller, the subjects' legs were dirtier, and, owing to the nature of the undergrowth in the test area, scratches from thorns and sharp-edged leaves were numerous enough to confuse the count. Leeches were therefore counted as "fed" if they were either attached, or detached but engorged, and this probably is a useful measure of the successful attacks or number of bites. Unfed leeches were classified as "alive" or "dead" since a number of unfed leeches were found dead but trapped in the socks or the rolled end of the trousers.

In a second set of tests, the bush-climbing Haemadipsa picta, which infested the vegetation and would readily transfer directly onto the volunteers from leaves or could drop on them, was studied. The ground-dwelling Haemadipsa zeylanica was also present. This experiment, which is summarized in Table VI, resolved itself into a comparison of treated uniforms with native dress.

From Table VI it is clear that treated uniforms offer significant protection. It is also shown that even with a reduced leech population, treated foot wear constitutes a major barrier to leeches.

TABLE VI. EFFECT OF M-1960 REPELLENT TREATMENT ON CLOTHING[14] - AGAINST TERRESTRIAL LEECHES

	With Treated Clothing				With Native Dress, All With Bare Feet & Legs			
	With Socks		With Bare Feet					
	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
	0	1	3	0	3	0	2	0
	1	0	1	0	5	0	2	0
	1	1	2	0	1	0	2	0
	0	0	2	0	0	0	3	0
	0	0	2	0	3	0	3	0
	1	0	1	0	3	0	3	0
Total	3	2	11	0	15	0	15	0
Mean	0.5	0.33	1.83	0	2.50	0	2.50	0

It was also observed that Haemadipsa zeylanica, when placed on a uniform sleeve treated with M-1960, would show every sign of distress; it would refuse to hold with its suckers, and would wriggle and throw out mucus. If it was not removed or did not escape as a result of its struggles, it would become limp after 5 or 10 minutes and would not recover.

Both repellent smell and repellent effect toward leeches persisted in uniforms subjected to one or two washings with soap and cold water, as mentioned above. In order to study how long this repellency would persist in the same clothing used in the experiment reported in Table IV, the clothing was washed three additional times with soap and cold water, thus making a total of five washes and rinses. When these uniforms were used to study aquatic-leech repellency, it was found that there was little difference between the treated and untreated clothing. However, after six washes and three periods of wear, the treated clothing still retained a measurable ability to repel terrestrial leeches although the repellency was not sufficient to afford complete protection under the conditions of the experiment. If the experiment had been conducted so that the trousers were tucked in the socks, the over-all repellency demonstrated would probably have represented complete protection.

The fact that the M-1960-treated uniforms, after several washings, proved ineffective in repelling aquatic leeches but retained a measurable repellency against terrestrial leeches may be explained by the habits of the leeches. The aquatic leech swims straight to the point of attachment, such as the exposed skin, and has little power to penetrate cloth. In these experiments, therefore, they would have had only slight contact with the treated uniforms, and only a repellent which would dissolve in the water and have an effect at a distance would afford complete protection; if the uniform covering was complete, even without chemical additives it would protect against aquatic leeches. The terrestrial leech, on the other hand, walks to the point of attachment and may have to traverse a considerable distance of treated cloth, to which they must attach with their suckers, in order to reach exposed skin. Therefore, a very slight repellency, incorporated in the clothing would be sufficient to discourage the terrestrial leech.

It is clear from the experiments reported that a large part of the effectiveness of the treated uniforms stems from the mechanical barrier provided by the cloth.

On the basis of the data collected, there is a strong suggestion that the impregnated socks were effective in repelling aquatic-leech attack; and, moreover, that the protection was equally effective for the legs bearing untreated socks. In the course of trying to use the same technique to repel the ground-dwelling Haemadipsa zeylanica, the treated socks seemed to act both as a repellent and as a trap for killing leeches which managed to break through the barrier provided by the socks.

Harrison, Audy, and Traub[10] continued studies on articles of clothing and footwear impregnated with the M-1960 all-purpose repellent to attempt to find articles which the natives could use to repel attacks of Hirundinaria manillensis, Haemadipsa zeylanica, and Haemadipsa picta. Their first study involved footless stockings, or puttees, impregnated with M-1960 repellent. This investigation was based on the impression that most leech bites were on the leg or ankle, but this proved to be false; in fact, most of the bites were found on the feet and toes, between the toes being a favorite site. The footless stockings, therefore, served merely to further concentrate the attack on the feet by discouraging the leeches from climbing higher. Their second study utilized socks covering the entire foot and ankle. The socks used were thick, knitted woolen ones of standard U.S. Army issue and were impregnated with M-1960 repellent. These socks, when untreated, could be readily penetrated by leeches, while the untreated thin nylon socks used in the earlier test of uniforms[14] offered a useful mechanical barrier. The treated knitted wool socks proved to be of little value against aquatic leeches, but were effective against terrestrial leeches.

Traub, Wisseman, and Audy[17] conducted additional studies on the M-1960 repellent; this work was incidental to their basic studies on the natural history of leptospirosis and scrub typhus. The organism which was used in this investigation was the ground-dwelling Haemadipsa zeylanica found in North Borneo at Lumu-Lumu. The Haemadipsa zeylanica is particularly abundant; despite constant vigilance on the part of the native volunteers, the leeches became attached to them and, at times, as many as 15 were observed per individual. The wearing of untreated socks and trousers as mechanical barriers did not appreciably decrease the incidence of attachment. The additional clothing, in fact, made it more difficult to detect the leeches prior to engorgement. Two Dusuns were provided with untreated Army shoes but with socks and trousers impregnated with M-1960; they remained free from leeches in the course of walking for 1/2 hour in vegetation observed to be harboring leeches. In contrast, two other Dusuns, who served as control subjects, wore their normal attire, i.e., shirts and shorts; each of these had 30 leeches attached to him after the same exposure. The repellent effect of treated trousers was found to be completely eliminated by covering the trousers with a set of plastic leggings; exposure for 10 minutes in an infested area resulted in 15 to 20 leeches crawling on the plastic surfaces.

At no time did the repellent M-1960 cause any irritation to the skin or any other toxic manifestations under these field conditions.

M-1960-treated uniforms washed four to five times with soap and cold water, or worn during and after a heavy rain, still retained effective repellent action toward terrestrial leeches.

Repellency of M-1960 Constituents and Other Compounds

In unpublished work by Traub[18], the comparative effectiveness of the constituents of the M-1960 repellent, diethyl toluamide, and n-butyl-4-cyclohexene-1,2-carboximide against terrestrial leeches was investigated. Table VII presents the compounds and concentration used; Tween 80 was not utilized since it was not known to have any repellent effect.

TABLE VII. COMPOUNDS AND CONCENTRATION[18]

Compound	Concentration
M-1960 Constituents:	
n-butylacetanilide	37.5 grams*
2-butyl-2-ethyl-1,3-propanediol	50 grams*
Benzyl benzoate	50 grams*
n-butyl-4-cyclohexene-1,2-carboximide	50 grams*
Diethyl toluamide	Applied directly to skin

*Compound emulsified into 1 gallon of water.

The tests were conducted in secondary-type jungle in the Kapit District, Sarawak. The leeches which are common in this area are *Haemadipsa zeylanica* and *Haemadipsa picta*.

As shown in Table VIII, these field trials indicated that the 2-butyl-2-ethyl-1,3-propanediol and benzyl benzoate were both highly repellent against leeches. Volunteers wearing uniforms impregnated with either of these ingredients, even when the uniforms had been washed four times after impregnation, sustained only a few leech bites. The n-butylacetanilide and n-butyl-4-cyclo hexene-1,2-carboximide were ineffective even when freshly applied. The diethyl toluamide when applied to the skin was extremely repellent to leeches and equal to the best constituents of M-1960 as shown in Table VIII. This compound, however, is unfortunately highly soluble in water; and when the skin was washed or the clothing to which the chemical had been applied was rinsed in water most of the repellency of this compound was lost.

The most recent research reported on leech repellents was conducted by Buu-Hoi[16] in Vietnam. The forests in South Vietnam, as most of those situated in regions with a climate and humid conditions of a similar type, are infested by different species of terrestrial leeches of the *Haemadipsa* family. The specific organism involved in this study was not identified. The tests were conducted at the coal mines of Nong-son, near Tourane, a region most heavily infested by leeches. In the first experiment, the M-1960 in solution was used to impregnate uniforms. Two effects on the terrestrial leeches were observed: (1) a repellent effect, measured by the much lower number of terrestrial leeches falling on the impregnated uniforms as compared with that on the untreated control clothing, and (2) a local effect in that the leeches did not succeed in clinging at their points of impact. The first effect was attributed to the chemicals 2-butyl-2-ethyl-1,3-propanediol, benzyl benzoate, and n-butylacetanilide, because of

TABLE VIII. RESULTS OF REPELLENT PERSISTENCY IN FIELD TRIALS AGAINST TERRESTRIAL LEECHES USING M-1960, CONSTITUENTS OF M-1960, AND N-BUTYL-4-CYCLOHEXENE-1,2-CARBOXIMIDE CLOTHING IMPREGNANTS, AND DIETHYL TOLUAMIDE SKIN REPELLENT[18] (FOUR TRIALS IN EACH TEST SERIES, WITH 6 VOLUNTEERS PER CATEGORY)

Compound	Type of Clothing	Number of Washings	Combined Numbers of Leeches and Bites					
			Total	Range	Mean	Standard Deviation	Protection Index*	Per Cent Reduction
First Test Series								
Diethyl toluamide	Uniform	0	2	0-1	0.1	0.3	145	99+%
2-butyl-2-ethyl-1,3-propanediol	Uniform	1	0	0	0	--	∞	100%
Benzyl benzoate	Uniform	1	0	0	0	--	∞	100%
M-1960	Uniform	1	0	0	0	--	∞	100%
Control (Untreated)	Uniform	1	309	9-21	13	3.5	--	--
Second Test Series								
Diethyl toluamide	Indigenous**	0	0	0	0	--	∞	100%
Diethyl toluamide	Indigenous**	1	323	9-19	15	4	1.3	20%
Control (Untreated)	Indigenous**	--	408	11-24	17	3	--	--
Control (Untreated)	Uniform	1	289	7-21	12	4	--	--
Third Test Series								
n-butylacetanilide	Uniform	1	240	5-15	10	2.7	1.2	18%
n-butyl-4-cyclohexene-1,2-carboximide	Uniform	1	262	7-16	11	2.8	1.1	10%
M-1960	Uniform	1	0	0	0	--	∞	100%
Control (Untreated)	Uniform	1	291	7-21	12	3.2	--	--
Fourth Test Series								
2-butyl-2-ethyl-1,3-propanediol	Uniform	4	19	0-4	0.9	1.2	17	94%
Benzyl benzoate	Uniform	4	22	0-3	1	1.1	15	93%
M-1960	Uniform	4	19	0-4	0.9	1.2	17	94%
Diethyl toluamide	Uniform	1	250	7-15	10	2	1.3	22%
Control (Untreated)	Uniform	4	321	8-20	13	3.5	--	--

*Protection Index: Total number of leeches by controls divided by number collected by volunteers with a particular repellent.

**These volunteers did not wear treated clothing, but applied the particular repellent to their bare skin.

their relatively high vapor tension. The second effect may be partly ascribed to Tween 80, which has no noticeable vapor tension. According to this experiment, protection was complete inasfar as the portions of the body which were covered by the uniforms.

Other series of tests by Buu-Hoi were similar to those conducted by Traub[18] and involved utilizing the constituents of M-1960 to study the repellency of the individual compounds. First, mixture of 400 grams of 2-butyl-2-ethyl-1,3-propanediol, 300 grams of benzyl benzoate, 200 grams of n-butylacetanilide, and 100 grams of Tween 80 was emulsified in 11 liters of water. This emulsion was used to impregnate uniforms as well as to coat uncovered skin areas. The protection provided was complete. However, some very slight skin irritation caused a little itching in some of the volunteers. Subsequently, tests were conducted under similar conditions with each one of the above substances used in the presence of Tween 80. In each case, the repellent effect obtained was less significant than in the previous tests involving the mixture of the three components plus Tween 80. Buu-Hoi concluded that the action of this repellent comprises two different effects: (1) a repellent effect stemming from the vapor tension of the substances composing M-1960, these substances exerting much more of an effect when they are used together than separately; and (2) an inhibiting effect on adherence of the leeches to the clothes or to the skin, this effect being possibly a result of the presence of the non-ionic emulsifier Tween 80.

A laboratory study on Hirudo medicinalis was conducted during the early stages of World War II by Wilson[12], beginning in the fall of 1943. According to the original plan, aquatic and terrestrial leeches were to be obtained along with colonies of American leeches for the study. Because of the delay in obtaining equipment and the organisms, it became impractical to utilize the tropical species. It was found possible, however, to obtain European medical leeches, Hirudo medicinalis, from a U.S. supplier. The test procedure consisted of placing the leeches in covered pint jars containing small amounts of water where they behave much like terrestrial leeches, hanging on the sides of the jar above the water; at the slightest agitation, they wave their heads about and actively seek a host. Dimethyl phthalate, Rutgers 612, and Indalone were tried as leech repellents. Fresh applications of the repellents were coated on the arms of subjects who then placed their coated arms over the openings of the jars. It was found that the leeches would release any holds and drop as soon as they touched any of these materials. Old applications of the repellents were found to prevent attachment of the leeches even after mosquitos were no longer effectively repelled.

Dimethyl phthalate (DMP), dibutyl phthalate, Rutgers 612, and 5 per cent dichlorodiphenyltrichlorethane in kerosene were investigated by Ribbands[13] as possible leech repellents. The Haemadipsa sylvestris leeches were not sufficiently abundant for field testing, so a laboratory procedure was devised. The technique consisted of using four strips of khaki uniform material, the strips being 12 inches long and of varying widths. The strips were impregnated with the substance to be tested and were then placed flat on damp bed sheets so that they enclosed an open rectangle. The leeches were confined, one at a time, within these rectangular areas, and the efficiency of the repellent was judged by the proportion of successful attempts to cross the impregnated strips. The first series of experiments was conducted in the open, and the leeches appeared to make every possible attempt to escape from the unfavorable environment of the white sheet; thus, their incentive to cross the impregnated strips to safety was considered to be at

least as great as their natural urge to feed. When moving on an untreated surface, the leech normally fixed its head sucker wherever it first touched; but when confronted with an impregnated surface, the sucker was withdrawn and re-oriented to another spot, and this process was continued until an untreated spot was reached. Table IX indicates the results of one series of experiments.

TABLE IX. COMPARISON OF STRIPS OF DIFFERENT WIDTHS IMPREGNATED WITH DIMETHYL PHTHALATE[13] (0.5 cc PER 12-IN.-LONG STRIP)

	Width of Strip			
	1/2 inch	3/4 inch	1 inch	1-1/2 inches
No. of attempts	50	50	50	50
Dry treated strips: times crossed	26	21	7	0
Wet treated strips: times crossed	2	--	0	0

The minimum effective barrier width for complete repellency, using dry materials, was 1-1/2 inches. When the treated cloth strips and surroundings were wet, however, a narrow or width sufficed. This was probably a result of the dimethyl phthalate spreading as a surface film over the wetted areas near the strips and thereby enlarging the effective strip width. It was found that the minimum completely effective dose of dimethyl phthalate corresponded to 0.5 cc per 12-inch by 1-1/2-inch strip, but that much smaller doses gave a very high degree of repellency. With a dose of only 0.0075 cc, 82 per cent of all attempts to cross failed and 97 per cent of all touches by the head sucker led to withdrawal.

The effect of washing on dimethyl phthalate repellency was also investigated. To test this effect, dimethyl phthalate-impregnated strips were laundered. The washing procedure for each strip was as follows: the strip was rubbed three times with moist soap, rolled ten times in the hands, dipped into water, rinsed ten times in clean water, removed, squeezed dry, twice rinsed in water, and squeezed dry. Each strip was tested by two of the leeches after each washing. The results of these tests are given in Table X.

TABLE X. EFFECT OF WASHING ON DIMETHYL PHTHALATE REPELLENCY[13]

	Percentage of Repelled Attempts			
Dose, cc	After 1st Wash	After 2nd Wash	After 3rd Wash	After 4th Wash
0.03	Nil	--	--	--
0.125	100	Nil	--	--
0.5	100	100	73	Nil
2.0	100	100	100	Nil

Since troops may be exposed to moisture such as rain, swamp water, and perspiration for considerable periods, a test was devised to determine the persistency of dimethyl phthalate repellency. Treated khaki strips were placed in very slowly running water and tested by means of two leeches at hourly intervals. Table XI shows the results obtained.

TABLE XI. EFFECT OF SLOWLY RUNNING WATER ON DIMETHYL PHTHALATE-TREATED STRIPS[13]

Dose, cc	Percentage of Repelled Attempts After 1 Hour	After 2 Hours	After 3 Hours	After 4 Hours
0.125	Nil	--	--	--
0.5	100	Nil	--	--
2.0	100	100	60	Nil

Dibutyl phthalate was found to be almost useless as a leech repellent. Khaki strips impregnated with 2.0 cc of dibutyl phthalate showed that this substance was only 62 per cent effective immediately after treatment. The strips were completely ineffective after 1 day in the sun, after one washing, or after 1 hour of exposure to running water. At a concentration of 0.125 cc, Rutgers 612 was at least as effective as the dimethyl phthalate at the time of application, but its effect wore off much more rapidly. Khaki strips treated with 0.125 cc of Rutgers 612 were only 5 per cent effective after being kept for 24 hours in the shade, and strips treated with 2.0 cc were completely ineffective after one washing or 1 hour in slowly running water. Dichlorodiphenyltrichlorethane (5%) in kerosene was completely ineffective at a 0.5-cc concentration per strip. Impregnation of this compound at 2 cc per strip was only 35 per cent effective, although the leeches traversing the material did behave abnormally after crossing.

Ribbands[13] concluded that dimethyl phthalate is a very effective terrestrial-leech repellent, but that the duration of its efficiency varies considerably with prevailing conditions such as the extent of exposure to sun light, heavy rain, swamps, and also the amount of laundering. Exact quantitative estimates of the relative importance of these different factors and of the reduction of the repellency of the dimethyl phthalate cannot be made. But, the results from the impregnation of strips with 2.0 cc of dimethyl phthalate can be taken as a guide: 4 hours of exposure to very slowly running water was equivalant to four launderings with soap and water, or to 9 days of exposure to moderate sunlight, or to 18 days of exposure in the shade.

For personnel walking through short grasses, full protection from terrestrial leeches may be obtained by the application of a thin smear of dimethyl phthalate as a 1-1/2-inch-wide band around the neck of shoes or boots and to the tongue and lace holes of the boots.[13]

Wherever possible, dimethyl phthalate or other repellents should be applied to parts of the clothing which are least exposed to the elements.[13]

Dimethyl phthalate was proved by Ribbands[13] to be not only a very effective, but also a very durable terrestrial-leech repellent. A dose of 4 cc per square foot applied to cloth in bands 1-1/2 inch wide was completely repellent under favorable circumstances for 6 days. A dose of 0.06 cc per square foot was more than 60 per cent effective immediately after similar application.

A method of protecting personnel who normally go bare-legged or bare-footed is the use of repellent liquids or ointments applied directly to the skin. This method is used to a certain extent by the people of Borneo and Malaya[4,10] who frequently smear their legs with oil of some kind or with tobacco juice.

A number of different ointments were tested including:

(1) Dimethyl phthalate (90%) + Santocel C (10%)
(2) Ethyl-beta-phenyl hydracrylate (88%) + Santocel C (12%)
(3) A mixture of dimethyl phthalate, 4 parts, Rutgers 612, 3 parts, and dimethyl carbate, 3 parts (85%) + Santocel C (15%).

In the first test of the effectiveness of these ointments against aquatic leeches, the ointments were applied to the right leg only of each volunteer, the left leg being left untreated. Approximately 10 grams were applied to the leg, including both the foot and ankle, by one of the investigators. The results are shown in Table XII.

TABLE XII. EFFECTIVENESS OF OINTMENTS AGAINST AQUATIC LEECHES[10]

Ointment	Treated Right Leg		Untreated Left Leg		Comparison With Zinc Oxide	
	Leeches	Bites	Leeches	Bites	t*	P*
Dimethyl phthalate	27	8	38	7	1.53	0.15
Ethyl-beta-phenyl hydracrylate	11	5	86	24	2.88	0.02
Dimethyl phthalate, Rutgers 612, and dimethyl carbate	23	7	44	11	1.97	0.07
Zinc oxide	56	18	66	32	--	--
Treated and untreated legs of ethyl-beta-phenyl hydracrylate group	--	--	--	--	2.1	0.06
Untreated legs of those treated with DMP and zinc oxide	--	--	--	--	1.47	0.18

* t, from the test recommended by Fisher and Yates[19] for determining the significance of the difference in this study between the total number of bites (both legs) on the volunteers under test and the controls, with P being the probability of obtaining such a difference by chance.

None of the ointments could be regarded as particularly effective against aquatic leeches since nearly all of the treated legs bore leeches and over half of them showed bites.

Scoring for the number of leeches and number of bites on the treated as compared with the untreated control legs gives the ethyl-beta-phenyl hydracrylate as the most efficient repellent, with the difference being statistically significant. If the total number of leeches on the volunteer, on both treated and untreated legs is taken into account, however, the dimethyl phthalate composition and the dimethyl phthalate, Rutgers 612, dimethyl carbate, and Santocel C mixture show as slightly better although here the differences are not statistically significant. It is a possibility that the dimethyl phthalate or one of the constituents of the mixture dissolves in the water and repels the leeches not only from the treated surface, but from the whole vicinity of the treated leg. Another possibility is that the leeches which come into contact with the dimethyl phthalate or the mixture are made acutely uncomfortable and lose the desire to feed. The authors concluded that these ointments are not of much value for protection against aquatic leeches.

The ointments were also tested against terrestrial leeches. Table XIII summarizes the results. The dimethyl phthalate and the dimethyl phthalate, Rutgers 612, dimethyl carbate, and Santocel C mixture showed a clear advantage over the ethyl-beta-phenyl hydracrylate; other factors being equal, a poorly applied coating of the latter will be less effective than a poorly applied coating of either of the other two test materials.

TABLE XIII. EFFECT* OF OINTMENTS ON REPELLING TERRESTRIAL LEECHES[10]

	Ointments				
	Dimethyl phthalate	Ethyl-beta-phenyl hydracrylate	Dimethyl phthalate, Rutgers 612, dimethyl carbate, and Santocel C	Zinc oxide	Control**
Legs:					
Treated (7 & 6)	0	0	1.1	1.0	4.0
Untreated (3 & 2)	1.7	5.0	9.3	2.5	1.5
Feet below:					
Treated legs	3.1	6.1	4.1	6.2	3.3
Untreated legs	10.0	5.0	5.7	10.0	6.0

* Summary results on 23 volunteers treated, some on both legs and some on one leg only (not feet). Figures are mean total numbers of attached leeches in 4 counts per leg (number of legs in parentheses), and mean numbers per foot below the treated or untreated legs.

** Local remedy used as a panacea for every ill.

It appeared from the tests described that a number of leeches broke through the protection of the ointment, and it was noticed that this breakthrough was more frequent as the tests progressed. The persistence of the mixture ointment against aquatic leeches was checked and is reported in Table XIV.

From the data in Table XIV, it appears that the good initial protection offered by the mixture ointment soon broke down, perhaps by the mechanical removal of the repellent by the water and mud. Similar slow breakdown of repellency was shown against terrestrial leeches. Table XV presents figures reclassified from other data, as total number of leeches seen within the times indicated.

TABLE XIV. PERSISTENCE OF DIMETHYL PHTHALATE, RUTGERS 612, DIMETHYL CARBATE, AND SANTOCEL C MIXTURE AGAINST AQUATIC LEECHES[10]

	Total Number of Leeches					
	Both Legs Treated		Right Leg Only Treated		Both Legs Untreated	
	Right Leg	Left Leg	Right Leg	Left Leg	Right Leg	Left Leg
First 1/2 hour, 2 counts total	4	4	1	15	24	29
Next 3/4 hour (1/2 to 1-1/4 hour), 3 counts total	18	14	15	25	19	17
After a 4-mile walk, one 10-minute exposure, 3 hours after application of ointment	11	5	14	18	12	23

TABLE XV. PERSISTENCE OF OINTMENTS AGAINST TERRESTRIAL LEECHES[10]

		Total Number of Leeches			
Time After Application, hour	Period of Exposure, hour	Dimethyl phthalate (11 legs)	Ethyl-beta-phenyl hydracrylate (11 legs)	Dimethyl phthalate, Rutgers 612, dimethyl carbate (11 legs)	Untreated Control (15 legs)
1-1/4	1/2	3	1	2	39
1-3/4	1	5	8	1	39
2	1-1/4	7	7	8	33
2-1/2	1-3/4	14	14	16	38

If an ointment is to be worth the trouble and expense, it must provide almost complete protection. Against aquatic leeches, none of the ointments tried was sufficiently effective to be worth while, but fortunately, mechanical barriers are effective. Against terrestrial leeches, the ointments were of considerably more value, although the repellency seemed to break down with 1 hour of use.

M-2065 and M-2066 Repellents

Walton, Traub, and Newson[11], in the summer of 1953, investigated the efficiency of the clothing impregnants M-2065 and M-2066 against terrestrial leeches in North Borneo. The M-2065 and M-2066 were experimental mixtures that were being developed by the U.S. Department of Agriculture under an Army-sponsored project as effective repellents against mosquitoes, mites, ticks, and fleas. Table XVI provides the chemical analysis of these compounds.

TABLE XVI. CHEMICAL ANALYSES OF M-2065 AND M-2066 REPELLENTS

	M-2065	M-2066
Undecylenic acid	29.00%	29.66%
n-propylacetanilide	29.00%	29.66%
n-butyl-4-cyclohexane-1,2-carboximide	29.00%	29.66%
Lindane	3.00%	1.00%
Tween 80 (emulsifier)	10.00%	10.00%

The solution utilized to impregnate the uniforms consisted of 1 part of M-2065 or M-2066 diluted with 11 parts of water, at the rate of 1 gallon of the concentrate for 28 uniforms. After the repellent-impregnated uniforms had dried, they were washed once with soap and water and then rinsed three times in warm water before they were issued to the volunteers. New untreated uniforms, which were utilized as controls, were also washed once with soap and water and then rinsed three times to eliminate the size in the new cloth. The leech repellency of the uniforms was tested after this initial washing and then again after additional washings with soap and warm water. Twenty native volunteers were employed for the tests. Five wore uniforms impregnated with M-1960; five wore uniforms impregnated with M-2065 or M-2066; five wore untreated uniforms; and five were attired in native dress, which consisted of only a light shirt and shorts, with no clothing on the legs and feet.

The uniforms were standard U.S. Army tropical issue consisting of light-weight jackets and trousers. The volunteers wearing the uniforms were also provided with tee shirts and cotton rayon socks; they wore the trousers tucked into the top of the socks and their footwear consisted of the open-work rubber sandals commonly worn in this area.

Studies were also made to determine the effectiveness of treated socks alone as a means of protecting the natives in this region. In these studies, five natives were bare footed, five wore untreated socks, five wore socks treated with M-1960, and five wore

socks treated with M-2066. All but the first group wore the open-work rubber sandals. The native volunteers were divided into five teams of four persons each, with each of the four types of clothing represented on each team. The leeches on the clothing and bodies of the volunteers were counted at the conclusion of each exposure period of 30 minutes. The teams were exposed in similar terrain two or three times during the course of each experiment. The investigators reported excellent protection from Haemadipsa zeylanica and Haemadipsa picta by all three repellents after the initial washings. A maximum of 1 leech was found on protected individuals; those wearing untreated uniforms averaged more than 14 leeches each; and those in native dress averaged more than 9 in one series of experiments and more than 22 in another.

After four additional washings with soap and warm water, M-1960 and M-2065 retained some of their leech repellency. Under these conditions, the average number of leeches on the persons wearing treated uniforms was less than one-fourth of the number of leeches observed in the control groups, but protection was no longer complete and individuals in both groups suffered leech bites. Table XVII and XVIII provide the results of these studies.

It was noted that many of the leeches found on the subjects in these experiments were unattached and had not fed, as indicated in Table XIX. The number of leech bites was utilized as a criterion of leech repellency since it is believed that the actual number of leeches attacking the control group was greater than the tables indicate. This was based on evidence that some leeches had completed their feeding before the end of the 30-minute exposure period and had dropped to the ground.

In the investigation of natives wearing impregnated socks, it was noted that excellent protection against terrestrial leeches was provided by the repellents. Only one leech fed on a native wearing treated socks. In contrast, 24 bites were noted on persons wearing untreated socks, and the bare-footed members of the teams suffered 70 bites. Since this experiment was conducted on a dry day, this technique of using impregnated socks would be expected to afford little protection against leeches attached to vegetation at a position above the knee-length socks. The investigators point out that the mechanical barrier provided by the untreated uniforms and socks was probably not as great as is indicated by the data in the tables. The presence of a relatively large number of unfed leeches on these persons was undoubtedly a result of the barrier and also of the relatively short period of exposure. It was observed that if the leeches were given sufficient time, practically all of them would have been able to feed.

The effective protection of the scantily clad natives by using impregnated socks is noteworthy. Because of the necessity for utilizing a large number of native porters, laborers, and guides in such regions, the habits of the people, the high cost of supplying impregnated uniforms, and the climate, the outfitting of such persons with complete sets of protective clothing is impractical. On the other hand, the cost of supplying impregnated socks would be insignificant. The experience of these investigators[11] proved that these natives would readily accept the practice of wearing socks with their sandals. In fact, as soon as the value of the impregnated socks became apparent in these experiments, socks became prized possessions, preferred to money and other items offered as wages. The impregnated socks, however, would be mainly effective against Haemadipsa zeylanica; Haemadipsa picta would be repelled only during dry seasons.

TABLE XVII. EFFECTIVENESS OF M-2065 AND M-2066 IMPREGNATION COMPARED WITH M-1960 IMPREGNATION - AGAINST TERRESTRIAL LEECHES

Type of Compound and Clothing	Number of Washings in Soap and Water	Number of Replications		Number of Leeches: Test Compound	M-1960	Untreated Uniforms	Native Dress
I. Complete uniform impregnated with M-2065	A. One: followed by three rinses in water	3	Total	1	0	77	48
			Range	0-1	0	9-27	9-13
			Mean	--	0	15.4	9.4
	B. As in "A" but with four additional washings	2	Total	15	16	70	54
			Range	1-6	1-9	4-32	10-12
			Mean	3.0	3.2	14	10.8
	C. As in "A" but with six additional washings	2	Total	24	9	37	53
			Range	1-12	0-3	5-10	9-13
			Mean	4.8	1.8	7.4	10.6
II. Complete uniform impregnated with M-2066	D. One: followed by three rinses in water	2	Total	2	2	71	114
			Range	0-1	0-1	8-24	22-26
			Mean	0.4	0.4	14.2	22.8
	E. As in "D" but with six additional washings	2	Total	37	12	32	63
			Range	4-11	0-9	2-10	10-15
			Mean	7.4	2.4	6.4	12.6
III. Cnly cotton-rayon socks impregnated with M-2066	F. One: followed by three rinses in water	2	Total	1	1	36	71
			Range	0-1	0-1	4-10	12-16
			Mean	0.2	0.2	7.2	14.2

TABLE XVIII. AVERAGE NUMBER OF TERRESTRIAL LEECHES ATTACHING IN 30 MINUTES TO VOLUNTEERS WEARING IMPREGNATED OR UNTREATED CLOTHING[11]

Type of Compound and Clothing	Number of Washings in Soap and Water	Number of Replications	Average Number of Leeches			
			Test Compound	M-1960	Untreated Uniforms	Native Dress
I. Complete uniform	A. One: followed by three rinses in water	3	0.06	0	5.1	3.2
	B. As in "A" but with four additional washings	2	1.5	1.6	7.0	5.4
	C. As in "A" but with six additional washings	2	2.4	0.9	3.7	7.1
II. Complete uniform impregnated with M-2066	D. One: followed by three rinses in water	2	0.2	0.2	7.1	11.4
	E. As in "D" but with six additional washings	2	3.7	1.2	3.2	6.3
III. Only cotton-rayon socks impregnated with M-2066	F. One: followed by three rinses in water	2	0.1	0.1	3.6	7.1

TABLE XIX. TOTAL NUMBERS OF FED AND UNFED TERRESTRIAL LEECHES PER GROUP OF VOLUNTEERS WEARING IMPREGNATED OR UNTREATED CLOTHING[11]

Type of Compound and Clothing	Number of Washings in Soap and Water	Number of Replications	Condition of Leeches	Number of Leeches: New Compound	Number of Leeches: M-1960	Number of Leeches: Untreated Uniforms	Number of Leeches: Native Dress
I. Complete uniform impregnated with M-2065	A. One: followed by three rinses in water	3	Unfed	1	0	43	1
			Fed	0	0	34	47
	B. As in "A" but with four additional washings	2	Unfed	11	11	50	0
			Fed	4	5	20	54
	C. As in "A" but with six additional washings	2	Unfed	18	7	20	2
			Fed	6	2	17	51
II. Complete uniform impregnated with M-2066	D. One: followed by three rinses in water	2	Unfed	2	2	32	8
			Fed	0	0	39	106
	E. As in "D" but with six additional washings	2	Unfed	25	5	19	5
			Fed	12	7	13	58
III. Only cotton-rayon socks impregnated with M-2066	F. One: followed by three rinses in water	2	Unfed	0	1	12	1
			Fed	1	0	24	70

The conditions under which these investigations were conducted were more stringent than the actual experimental procedure indicates. Since the volunteers were frequently exposed to heavy rains and were almost constantly walking through wet vegetation, their clothes were usually saturated with water most of the time during the experiments. Therefore, in effect, these garments were rinsed more often than indicated.

Current Leech-Repellent Program

The only known current effort in studying leech repellents is a program which has been recently initiated at the U. S. Department of Agriculture, Gainesville, Florida, by the U. S. Army, Limited War Laboratory, to investigate aquatic-leech repellents. The program is concerned with utilizing "off the shelf" formulations for aquatic-leech repellency. The repellents are to be applied directly to the skin rather than used as clothing impregnants. In addition to the repellent testing, a study will be made of the removal of leeches which are attached, methods of stopping the bleeding, and reduction of infection. The problem, according to Dr. Carroll Smith[8], is to be approached from a practical standpoint and will not involve a long-range study; this contract is for an 18-month period based on an 18-man-month rate of effort. Among the compounds to be studied is diethyl toluamide compounded with protective creams such as West Chemical Products Incorporated Silicone Protective Cream. Other compounds will be evaluated using an arm-washing test technique developed by the U. S. Department of Agriculture for testing insect repellents. This technique, as modified to study leech repellents, consists of immersing the arm treated with repellent in a circulating tank of water containing domestically available aquatic leeches. The number of bites will determine the effectiveness of the repellent. Dr. Smith indicates that they are having difficulty in obtaining a domestic aquatic leech which will draw blood from humans. Following the laboratory test phase, the compositions will be field tested. The emphasis of this program is on immediate repellent use rather than on a long-range repellent development or a study of leech morphology and taxonomy.

While the M-1960 repellent is effective if used against terrestrial leeches, it is less effective, if not completely ineffective, toward aquatic leeches according to Dr. Clyde Barnhart, Limited War Laboratory, who is the program monitor. The Gainesville study will therefore not concern itself with terrestrial leeches. The Gainesville group, previously at Orlando, Florida, has had extensive experience in evaluating chemicals as insecticides and repellents. Some 11,000 insecticide compounds have been tested in their laboratory, thus providing their personnel with considerable routine-testing experience which should be of value in their new study.

From a report from the Defense Development Exchange Survey Team[73], it was learned that the Research and Development Center, Camp Murphy, Quezon City, Luzon, Philippines, is giving priority consideration to an insecticide for leeches. Details are unavailable as to whether repellents or chemical poisons are being studied. Colonel Constancio Valesco, Chief of the Center, provided this information to the Survey Team.

Repellent-Testing Techniques

Table XX summarizes the methods used in the laboratory and field to evaluate leech repellents. It is important that standard methods for leech-repellency determination be developed so that the results obtained in field and in laboratory tests can be validly compared.

In field tests related to leech-repellent studies, native volunteers must be watched since they have a tendency to catch extra leeches and to put them in their pockets so as to watch them squirm in the repellent environment. The data obtained under these conditions would, of course, be incorrect. Also, since the natives try to please their employer, they answer questions with the answers which they feel are desired; such answers may not be interpreted appropriately by the untrained scientist working in underdeveloped areas.

A tin with moistened leaves and a semi-loose lid may be used to transport leeches for several days[7]. Glass jars with moistened cut turf, moss, and leaves may also be used. The lid of such jars may be a tightly tied cloth, the threads of which are close and strong[4].

In connection with the possibility of using rats as a host in the laboratory study of leeches, Harrison[7] points out that this would not be satisfactory. Caged rats detect and destroy attacking leeches.

TABLE XX. TESTING TECHNIQUES USED TO EVALUATE LEECH REPELLENTS

Type	Brief Description of Method	Reference
Field test	Treated and untreated socks used with bare legs and feet as control. Subjects pass through leech area, and number of bites, number of live leeches, and number of dead leeches counted.	10, 11
Field test	Treated and untreated uniforms used, with a count of the number of leech bites.	10, 16
Laboratory test	Repellent-coated arm placed over a bottle containing leeches, and the number of bites counted.	12
Laboratory test	Strips of material arranged to enclose a square or rectangle. Strips are of varying widths and represent various concentrations of repellent; impregnated strips placed on a flat damp bed sheet. Experiment conducted in the shade or in the sun. Number of attempts which leech makes before getting over repellent-impregnated strip is a measure of effectiveness of repellent.	13
Laboratory test	2 x 1-inch pieces of filter paper treated with 20 cubic millimeters of repellent per square inch. Paper, after drying for 3 hours, placed in path of leech. If 10 leeches did not cross over paper, but sought a way around it, test material was considered repellent.	4
Laboratory test	Aquarium containing leeches and with water circulating has a opening in which an arm coated with potential repellent is inserted. Number of bites are recorded. This technique is used with aquatic leeches.	8

COMPOUNDS MERITING STUDY

On the basis of the work uncovered to date, it appears that the studies on insect repellents may well be applicable to leech repellents. This is borne out in the case of M-1960, diethyl toluamide, and dimethyl phthalate in their effectiveness as terrestrial-leech repellents as well as their insecticide qualities. It is not known if the same will hold true relative to aquatic leeches and other terrestrial species. Experimentation on leech repellents, as with insect repellents, is extremely difficult because there are three entities involved[20] – the insect or leech, the site of attraction, and the repellent. Environmental conditions may modify the effect of each of these.

Laboratory studies on the attraction stimulus provided by dark objects would be of interest. While laboratory tests on mosquito repellents have become somewhat standardized, the problem still remains in the leech area to select for study one or a small number of organisms from the many species possible.

A substance which may not show up well as a repellent under laboratory testing may prove adequate when used on clothing in the field. This may explain the fact that although both benzyl benzoate and dibutyl phthalate have been reported on adversely in laboratory tests, they seem to provide adequate protection in the field[14].

Tests conducted by the U. S. Department of Agriculture[21] on the persistence of insect repellents consisted of subjecting a sock, freshly treated with each class of repellent, to one or more (up to 15) rinses in cold water and after each rinse testing the sock against yellow fever and common malaria mosquitoes. If the test material was still effective after the second rinse, the sock was washed for 15 minutes in hot, soapy water and then rinsed for 5 minutes. Only a small number of the insect repellents withstood the exposure to water. On the basis of these tests, the following insecticides and repellents are suggested as candidate compounds for leech-repellent evaluation:

Acetamide, n-amyl-alpha-butoxy-
Acetamide, alpha-butoxy-n-cyclohexyl-
Acrylic acid, p-methoxybenzyl ester
Aniline, n-butyl
Anisole, p-nitro-
Benzene, m-dinitro-
Benzoic acid, 0-(2,4-dinitrophenoxy)-
Caprylic acid, tetrahydrofurfuryl ester
Cinnamic acid, isobutyl ester
Cinnamic acid, methyl ester
Cinnamic acid, propyl ester
Citronellal, oxime
o-Cresol, 4,6-dinitro-
Crotonic acid, phenethyl ester
2,5-Cyclohexadien-1-one, hexachloro-
Cyclohexanecaproic acid
Cyclohexanepropionic acid, 2-methoxyethyl ester
Cyclohexanevaleric acid
Cyclohexanol, 2-cyclohexyl-
Cyclohexanol, 2-m-tolyl-, trans-

4-Cyclohexene-1, 2-dicarboximide, n-butyl-
2, 4-Decanediol
Ethane, 1-(p-tert-butylphenoxy)-2-(2, 4-dinitrophenoxy)-
Ethanol, 2-(p-sec-butylphenoxy)-
Ethanol, 2-dodecyloxy-
Ethanol, 2-[2-(2-ethylhexyloxy)ethoxy]-
Ethanol, 2-(p-ethylphenoxy)-
Ethanol, 2-(2, 5-xylyloxy)-
Ether, 2, 4-dinitrophenyl 4-methylcyclohexyl
Ether, 2, 4-dinitrophenyl propyl
Glutaric acid, n, n-diisopropyl-, ethyl ester
10-Hendecenamide, n, n-diethyl-
Hendecenoic acid
2, 4-Heptanediol, 5-ethyl-
Indalone. (2H-Pyran-6-carboxylic acid, 3, 4-dihydro-2, 2-dimethyl-4-oxo-, butyl ester)
Lauric acid, ester with 2-methyl-1, 3-dioxolane-4-methanol
1 (2H)-Naphthalenone, 3, 4-dihydro-5, 8-dimethyl-
Phenol, 2-sec-butyl-4, 6-dinitro-
Phenol, phenethyl-
Phenol, tetrachloro-
1, 2-Propanediol, 3-(2-ethylhexyloxy)-
1, 3-Propanediol, 2-butyl-2-ethyl-
2H-Pyran-6-carboxylic acid, 3, 4-dihydro-2, 2-dimethyl-4-oxo-, ethyl ester
Succinamic acid, n, n-diisopropyl-, propyl ester
Thymol, chloro-
3, 5-Xylenol, 4-chloro-
Heptylamine, n-heptyl-1-methyl-

Molluscacides may also be of some interest for consideration in a leech-repellent development program. Dobrovolny and Dobbin investigated 37 compounds in static water and found that primary phenyls and related compounds gave the most promising results against molluscs.[22] Chemicals which were 95 to 100 per cent effective in 2- to 5-ppm concentrations were pentachlorophenol, emulsifiable (7. 2% active); copper pentachlorophenate; sodium pentachlorophenate; 3, 5-dibromo-2, 4, 6-trichlorophenol; bis (2-hydroxy-3, 5, 6-trichlorophenyl) methane; monosodium salt of bis (2-hydroxy-3, 5, 6-trichlorophenyl mercuric acetate; and dinitro-o-cyclohexylphenol. These compounds were effective against snails bearing schistosomiasis; however, they also killed fish intermediates.

Another potential molluscacide[23] is composed of a carrier metaldehyde in an amount of 0. 1 to 10 per cent by weight and at least 0. 5 per cent by weight of a compound selected from the group consisting of chloralammonia, chloralhydroxylamine, trichloracetaldoxamine, arabo-chloralose, gluco-chloralose, manno-chloralose, galatochloralose, chloraurethane, chloral-formamide, monochloralcarbamide, and dichloralcarbamide.

The use of formulations containing the sodium salt of 3-trifluoro-methyl-4-nitrophenol against fish parasites such as the sea lamprey and leeches has been demonstrated in various environments[2]. Leeches were killed when 3 to 7.5 milligrams per liter of a solution of 50 per cent of the sodium salt, 30 per cent water, and 20 per cent $HC(:O)NMe_2$ were added to the water. Leeches are rapidly poisoned when powdered santonin was externally applied, as a result of the alkalinity of the external mucus of the leech which favors solution and absorption of santonin[25].

Because of the success of mosquito repellents, acting also as leech repellents, those insect skin repellents which have been shown to be effective should be considered, including: n, n-diethyl-m-toluamide; o-ethoxy-n, n-diethylbenzamide; o-chloro-n, n-diethylbenzamide; citral-malonic acid condensate # 1; and propyl-n, n-diethylsuccinamate. Studies should also be made of effective mixtures of 40 per cent of dimethyl phthalate, 30 per cent of 2-ethyl-1, 3-hexanediol, and 30 per cent of n, n-diethyl-m-toluamide, o-ethoxy-n, n-diethylbenzamide, or propyl n, n-diethylsuccinamate; hendecenoic (undecylenic) acid; 1-butyl-4-methylcarbostyril; amyl mandelate; and 30 per cent of hendecenoic acid, 30 per cent of n-butyl-4-cyclohexene-1, 2-dicarboximide, 30 per cent of n-butylacetanilide, and 10 per cent of emulsifier (Tween 80)[26].

Starnes and Garnett[27] have shown the insect-repellent value of Indalone and Crag Fly Repellent (butoxypolypropylene glycol) in connection with synthetic sweat. These compounds merit consideration as possible leech repellents because of their persistence.

Cross and Snyder[28], in a search for insect repellents which would be even more lasting than benzyl benzoate, found the following materials very promising: benzil, 2-thenyl benzoate, p-cresyl benzoate, diphenyl carbonate, and 2-thenyl salicylate. Diphenyl carbonate and 2-thenyl salicylate gave complete protection and benzil and 2-thenyl salicylate gave a high degree of protection through more days of aging than the other materials.

The tendency of sweat to impair insect repellency has been studied extensively at the Naval Medical Research Institute by Pijoan, Jachowski, and Gerjovich[29]. Their method involved testing repellents first in the laboratory by an initial screening for dry-skin repellency and then selecting the promising ones for further testing under simulated tropical conditions. These tests indicated that sweat is an important factor in decreasing repellency time and revealed the failure of heretofore acceptable insect repellents to operate efficiently under tropical conditions.

Since World War II, thousands of compounds have been tested as repellents against mosquitoes and biting flies. To date, no consistent correlation has been found between repellency and chemical formulation or configuration.[3] However, two promising groups are certain hydrogenated naphthol derivatives and hydrogenated diphenyls.[30] The mixing of these with 2-phenyl cyclohexanol results in a prolongation of insect repellency as a result of synergistic interaction. Three of the most effective mixtures which repel <u>Aedes aegypti</u> for longer than 289 minutes at environmental temperatures of 90 F dry bulb and 80 F wet bulb are: 2-phenyl cyclohexanol (washed) plus 2-naphthol, 1, 2, 3, 4-tetrahydro, and acetylglycine ester; 2-phenyl cyclohexanol (washed) plus 2-naphthol, 1, 2, 3, 4-tetrahydro, and glycollic ether (crude); and 2-phenyl cyclohexanol (washed) plus 2-cyclohexyl cyclohexanol (washed). Since toxicological studies must be done on these materials, their use as repellents cannot be recommended as yet. However, these may have potential.

The solvent in which the insect repellent is mixed has an added effect on repellency.[3] As an example, dimethyl phthlate is more repellent when dissolved in hexyl alcohol than when dissolved in ethyl alcohol. This leads to the deduction that an insect repellent solution or mixture may be modified by (1) a quantitative reduction of repellent by dilution, (2) the repellent properties of a solvent, and (3) the repellent effect induced by the new physical and chemical properties of the solution. The incorporation of vinylite binders in insect repellents has lengthened the duration of uniform repellency, especially under adverse conditions of wear and laundering.

Entex (0, 0-di-Me-0-[4-(methylthio)-m-toly] phosphorothionate) combines a low mammalian toxicity with long residual effect on a wide range of insects and provides control of flies, mosquitoes, ticks, roaches, and lice which have become resistant to chlorinated hydrocarbons[31]. Hexamide[32] used as a 1 to 3 per cent H_2O emulsion may be also considered as a potential leech repellent since its value as a blood-sucking insect repellent has been demonstrated for domestic animals. Mel'nikov, Mandel'baum, and Lomakina[33] report that Indalone and dimethyl carbate [dime-cis-bicyclo (2. 2. 1) hept-5-ene-2, 3-dicarb-oxylate] are effective as repellents against flies and mosquitoes.

Pheromone[34] substances being secreted by organisms that influence the behavior of other organisms of the same or different species may be considered for possible study as leech repellents.

Insect-repellent wax compounds[35] made up of 0. 2 to 5% by weight of a lower alkyl ester of maleic, fumaric, or succinic acids, on the basis of total solids, waxes, resins, or plasticizers, may also be of potential interest as leech repellents. Specifically, a mixture of carnauba wax, 70 parts, ceresin wax, 30 parts, morpholine, 11 parts, NH_4OH, 5 parts, H_2O, 928 parts, and di-n-butylsuccinate, 1 part by weight, when evaporated, leaves a wax coating containing 100 mg of the ester per square foot. This mixture applied to wood panels provided a 100% repellency to roaches for 4 days. The use of repellency regeneration by polishing is a technique which should be considered.

Selected snake repellents[36] which are being investigated should also be studied as potential leech repellents.

Compounds such as those being investigated at the U. S. Army Tropical Research Medical Laboratory, Puerto Rico[37], relative to preventing penetration of the skin by cercariae of <u>Schistosoma mansoni</u> should also be considered in a leech-repellent evaluation study. In order to stabilize these compounds in the presence of water, they are combined with up to 20% by weight of Abbott's "Covicone" protective skin cream; this cream is described as a "special plasticized combination of silicone (dimethylpolysiloxane), nitrocellulose, and castor oil, suspended in a greaseless vanishing cream base". The use of such a base compound is predicated on the fact that there must be contact of skin with water if infection with schistosomes is to occur; therefore, any substance that would protect or prolong the activity of the test repellent in contact with water would have distinct advantages. Preliminary tests have shown that several preparations consisting of up to 20% repellent in "Covicone" afforded 100% protection against <u>Schistosoma mansoni</u> in mice for at least 3 hours, during which time there were three 15-minute contacts with water, alternating with 45-minute periods of normal activity on the part of the mice.

SENSITIVITY OF LEECHES TO CHEMICALS AND OTHER STIMULI

Both aquatic and terrestrial leeches have well-developed chemoreceptive senses. Some authors[38] have distinguished between taste and smell as "sensed" by aquatic leeches, i.e., the distinction between a response to chemical substances drifting in the water and the response to close contact between the leech and a particular substance. Terrestrial leeches have been only incidentally studied by Moore[39].

Kaiser[40] made a detailed study of the substances which evoke a reaction in Hirudo. His study consisted of placing five leeches in 200 cc of water in a dish, and then while the leeches were at rest he tested the effect of each substance by adding it, drop by drop. The leeches responded by making characteristic jerking and quivering movements when acids were added; various other substances evoked normal locomotory exploratory movements. His results are summarized in Table XXI.

TABLE XXI. RESPONSE OF HIRUDO MEDICINALIS TO CHEMICALS ADDED TO WATER[40]

Chemical	Concentration, %	Responded
Formic acid	0.02	Quivering reaction; left water
Acetic acid	0.04	Weaker reaction than above; after 5 minutes anterior sucker was out of water
Propionic acid	0.04	As for acetic acid
Iso-butyric acid	0.04	As for acetic acid
n-butyric acid	0.04	Weaker reaction; left water after 30 seconds
Oxalic acid	0.04	Typical acid reaction, as indicated above
Malonic acid	0.10-0.20	Typical acid reaction, as indicated above
Succinic acid	0.10-0.20	Typical acid reaction, as indicated above
Citric acid	0.02	Typical acid reaction, as indicated above
Hydrochloric acid	0.02	Typical acid reaction, as indicated above
Phenol	0.02	Shock reaction; left water
Naphthol	0.04	Typical acid reaction; left water
Quinine, Caffeine, Atropine, Cocaine, Morphine	0.02	Swam about restlessly; then came to rest with anterior sucker out of water
Ammonia	0.02	Strong reaction; left water
Indol	0.04	Left water hastily
Pyridine	0.02	Reacted only to undiluted substance
Camphor	0.02	No reaction

Aquatic leeches have chemoreceptors which provide information about substances in water, substances with which the anterior sucker comes in contact, and substances containing the blood which is passed through the buccal cavity. The chemoreceptors are confined to the head; thus, when this part of the body is out of the water, the leeches are no longer aware of a noxious stimulus. Terrestrial leeches also have chemoreceptors; however these have not been studied.

Other information on the sensitivity of aquatic leeches and leech muscle is presented in Table XXII. Where concentrations were cited, this information is included in the table. The reader will note obvious disagreements in responses in the literature cited. Some of these differences may be explained on the basis of the fact that leech muscle reacts much differently than the intact organism.

The sensitivity of the dorsal muscles of various species of aquatic leeches to acetylcholine was investigated by Negrete[43]. The investigation showed a great difference between species. This observation may well apply to the effectiveness of repellents toward different species. As pointed out previously, the location of chemoreceptors also plays an important role in sensitivity to chemicals.

Studies by Teitel and Dallmann[58] on the effects of potassium ions on the muscular system of leeches Haemopis sanguisuga, or Hirudo medicinalis reveal that, with concentrations between 0 and 0.48 g KCl per liter, a value corresponding to a quadruple physiological concentration in the frog-Ringer's solutions, the potassium ions exerted a relaxing and tonus-lowering effect. These end results agreed very well with the results obtained on the distribution and reciprocal exchange of the potassium and the sodium ions in the phase of activity or periods of rest on nervous or muscular fibers. From the point of view of this study, the relaxing effect of the potassium ions in physiological or in lower concentration is understandable since in this way the muscle fibers concentrate potassium inside their cells and can thus pass into the state of rest.

Increasing the number of methyl or hydroxy groups has no effect on the toxicity of diphenyl ether compounds against aquatic leeches. Only para-phenoxybenzoic acid has considerable toxicity.[59]

Leeches are very sensitive to metallic and narcotic poisons such as copper sulphate, zinc chloride, and nicotine and, under experimental conditions, may be killed by very dilute solutions.[60] The use of these under natural conditions has not been successful as the leeches escape injury by crawling or swimming away.

5-hydroxytryptamine was found to have an inhibiting effect on leech muscle.[47] It reduces the contraction produced by acetylcholine or nicotine and accelerates the relaxation of the muscle when the substances are washed out. This acceleration of relaxation allows a more rapid assay of acetylcholine in this preparation.

Moore[39] concluded from his study of terrestrial leeches that they have sense organs of touch, high perception, and taste-smell; all three of these sense perceptions are used to ascertain the presence and position of their prey. A hungry leech in the field tends to move upward (negative geotropism). Thus, leeches will climb to the top of any plant or object with which they come in contact; when at the top, they will attach their posterior sucker firmly, stretch their bodies to the utmost, and, with rather

TABLE XXII. RESPONSE OF LEECHES OR LEECH MUSCLE TO VARIOUS COMPOUNDS

Compound	Organism	Response	Reference
Acetylcholine	*Diplobdella brasiliensis*	Sensitive at concentration of 1.3×10^8	41
Acetylcholine	*Hirudinea muscle*	Sensitive	42, 43
Muscarine	Ditto	Ditto	41
Barium	"	"	41
Nicotine	"	"	41
Adrenalin	"	Insensitive to compound	41
Histamine	"	Ditto	41
Caffeine	Leech muscle	No effect	44
Phenol	Ditto	Increased tonic and rhythmic contractions	45
O-cresol	"	Ditto	45
M-cresol	"	"	45
P-cresol	"	"	45
a-naphthol	"	"	45
Thymol	"	"	45
Isothymol	"	"	45
Eserine	"	Intensified contractions produced by electrical stimulation	46
5-hydroxytryptamine	*Hirudo medicinalis* muscle	Reduced the contractions caused by acetylcholine or nicotine	47

TABLE XXII. (Continued)

Compound	Organism	Response	Reference
1-camphor	Leech muscle	In concentrations of 1:1000 to 1:15,000 produced stimulating actions	48, 49
d-camphor	Ditto	Ditto	48, 49
i-camphor	"	Stimulated rhythmic contractions	49
Quinine phenylethylbarbiturate	"	Ditto	50
Piperazine	Hirudo medicinalis	Tolerated high concentration	51
Anthistaminic drugs	Leech muscle	Acted spasmogenically	52
Orcinol	Species not identified	0.04% concentration killed in 300 minutes	53
6-orcinolcarboxylaldehyde	Ditto	0.04% concentration killed in 34 minutes	53
6-bromoorcinol	"	0.04% concentration killed in 67 minutes	53
5-chloroatranol	"	0.04% concentration killed in 11 minutes	53
2,4-dihydroxy-1-ethylbenzene	"	0.04% concentra- killed in 59 minutes	54
2,4-dihydroxy-1-propylbenzene	"	0.04% concentration killed in 11 minutes	54
2,4-dihydroxy-1-butylbenzene	"	0.04% concentration killed in 9 minutes	54

TABLE XXII. (Continued)

Compound	Organism	Response	Reference
2,4-dihydroxy-1-isoamylbenzene	Species not identified	0.04% concentration killed in 12 minutes	54
2,4-dihydroxyacetophenone	Ditto	0.04% concentration killed in 42 minutes	54
2,4-dihydroxybutyrophenone	"	0.04% concentration killed in 26 minutes	54
2,4-dihydroxyisovalerophenone	"	0.04% concentration killed in 24 minutes	54
2,4-dihydroxycaprophenone	"	0.04% concentration killed in 42 minutes	54
Hexylresorcinol	"	0.04% concentration killed in 17 minutes	54
Ammonium compounds	Leech muscle	NH_4 had an irritating action, followed by paralysis; $(NH_4)_2SO_4$ was not as irritating as NH_4CNS	55
Guanidine	Ditto	Strongly toxic	55
Thymol	"	Paralyzed the muscle	56
Green apiol	"	Caused a strong contraction	56
Mustard oil	"	Ditto	56

TABLE XXII. (Continued)

Compound	Organism	Response	Reference
Copper sulphate	*Hirudinaria manillensis*	Concentration of 1:1000 killed adult leeches in 2 to 2.5 hours; 1:50,000, in 48 to 75 hours; 1:100,000, harmless to adults, but killed young leeches in 48 to 57 hours; 1:500,000, killed young leeches in 10 days.	57
Sodium chloride	Ditto	2% concentration killed adult leeches in 48 to 75 hours; 3% concentration killed in 3 to 7 hours; 4% concentration killed in 1 to 2 hours; 5% concentration killed in 30 to 70 minutes.	58
Potassium ion	Leech muscle	Subphysiological concentration of K+ caused muscle to relax; concentration, followed by waves of contraction and a long lasting contraction.	41
Potassium chloride	*Diplobdella brasiliensis*	Contractions at concentration of 1:20,000.	41

violent movements, reach out in all directions. It is from this highest position possible and by reaching in this manner that it may be possible for them to come into contact with their prey.

He experimented with their sense of smell by watching them climb, since skin probably has a specific chemical that acts as an attractant to them. After they had been unsuccessful in coming in contact with him, they descended to the ground, moved to a closer stem, and repeated their climbing and stretching. After they had almost reached him, he carefully moved to a new position and noticed after some hesitation that they changed their course and still followed him. The Haemadipsa species thrives only on the blood of vertebrates; it has an enormous appetite although it feeds only occasionally. One feeding may suffice for several months, a year, or even a lifetime.

In general, leeches are strongly photonegative in their behavior toward light, some species being more so than others.[38,61] In the blood-sucking species, it is their need to obtain a meal that modifies their normal light-avoiding reactions. When a shadow passes over a leech in its natural habitat, it is likely that this is caused by the movement of a larger animal. The blood-sucking leech often reacts by making searching movements or even by swimming upwards through the water. The nonparasitic forms react by flattening themselves against the substratum or abruptly ceasing ventilatory movements. A 50 per cent reduction in the light falling on the leech will bring it to the attack position, so apparently a man's or animal's shadow is also an attraction.

Land leeches follow both a current of warm, moist air and the human breath; their line of progress toward the source may be deflected by altering the direction of the current. Since atmospheric air and breath were found to be equally attractive, it is unlikely that the latter contains a chemical attractant. Such behavior leaves little doubt that temperature acts as a token stimulus.[4] This sensitivity seems to be of a high order, for a response to gentle blowing through the cloth covers of bottles containing leeches could be detected from a distance of several feet.

Some leeches are able to locate the center of a disturbance in water.[38] Hungry leeches will converge on a center of disturbance in a pond in which they are living even if the disturbance is made by movement of a stick rather than by any part of a living animal.

Pieces of skin and their underlying connecting tissues, which have been cut from dead rabbits, cats, and rats, proved to attract terrestrial leeches and continued to act as a diminishing powerful attractant for several days.[4] The greatest interest for a point of attachment was the cut edges of the skin. Pieces of rabbit muscle and rabbit fat which were placed in the paths of leeches were traversed by them without any interest being shown. Pieces of rabbit skin stretched over a beaker containing warm water (at 40 C) and another over a beaker containing cool water (at 18 C) were studied. In each case, the leeches were attracted; however, in the former, with the water at 40 C, the leeches appeared to show a slightly greater interest.

Those leeches of the genus Hirudo and Theromyzone[38] that suck the blood of warm-blooded animals are stimulated to attach themselves to a warm object at 33 to 35 C. It was determined from these studies that once the sucking reflex begins, the leech is oblivious to the fact that it is not filling its body with blood. Leeches are not interested

in blood alone; when put in contact with freshly drawn blood, they passed their suckers through the film, but did not engorge the freshly drawn blood. It is an interesting fact that the young leech seems to possess a boring ability rather than a sucking ability as is common to its more mature relative; in animals, it is quite common to find small leeches under the skin at a considerable distance from the point of entry. [5]

Miller[62] conducted detailed morphological studies of the nervous and muscular system of the leech Haemopis marmoratis. On the basis of such knowledge and through the treatment of behavior patterns, it is possible to describe and explain certain phases of leech behavior. In order to complete a study of leech behavior, the physical explanation of the responses of the organism is necessary. To be able to do this, it is imperative that the nervous and muscular systems be well understood.

OCCURRENCE OF VARIOUS LEECHES

To give a full and accurate taxonomy and to describe the occurrence of the leeches of the world as far as they have been reported in the literature is beyond the scope of this report. Because of the number of genera and species which have been named without an adequate description having been provided, their taxonomy is further complicated. To determine the validity of the various names would require a long-term program which would have to be conducted in many parts of the world.

However, a survey of the occurrence of leeches would not be complete without a scheme of classification for them. Table XXIII presents four different classifications, as suggested by Mann[38], Harding and Moore[63], Faust, et al.[64], and Miller[65]. While similarities exist between the various classifications, it is evident that an extensive effort would be necessary to resolve the differences. For this study reported herein, the scheme suggested by Mann has been arbitrarily selected since it is the most recently (1962) developed taxonomy.

The most important blood-sucking leeches which attack man and domestic animals as included in Mann's Suborder Gnathobdellae are Family Hirudidae and Family Haemadipsidae. Several parasites of man and mammals are also classed in the Suborder Rhynchobdellae, Family Glossiphoniidae, Subfamily Haementeriinae. Table XXIV provides a summary of the parasitic forms and their habitats.

Terrestrial leeches are found chiefly in local aggregations or colonies. Such areas of concentration may be only a few feet or yards in diameter or they may cover acres, especially in forest grazing lands. Often, one may proceed for several miles along jungle trails which could be expected to harbor leeches, but none may be encountered.

It has been found that leech fauna increases when the CaO content of fresh water bodies is above 9 mg per liter.[66]

Coulter[5] in his study of leeches in Ceylon reported that heat and cold seem to have little effect on leeches, providing that adequate moisture is present in the atmosphere and soil. Altitude in Ceylon, however, seems to bear an important relation to the quantity of leeches since they appear to thrive in certain belts according to the height above sea level.

TABLE XXIII. DIFFERENT CLASSIFICATION SCHEMES FOR LEECHES

Mann[38]	Miller[65]	Harding and Moore[63]	Faust, Russell, and Lincicome[64]
ORDER Hirudinea	ORDER Rhynchobdella	Family Pisobdellidae	ORDER Gnathobdellida
Suborder Acanthobdellae	Family Glossiphonidae	Family Rhynchobdellae (Glossiphoniidae)	ORDER Rhynchobdellida
Suborder Rhynchobdellae	Family Icthyobdellidae	Family Herpobdellidae (Erpobdellidae)	ORDER Pharyngobdellida
Family Glossiphoniidae	ORDER Arhynchobdella	Family Hirudidae (Gnathabdellae)	
Family Piscicolidae (Ichthyobdellidae)	Suborder Gnathobdella		
Suborder Gnathobdellae (Arhynchobdellae)	Family Hirudinidae		
Family Hirudidae	Suborder Herpobdellida		
Family Haemadipsidae	Family Herpobdellidae		
Suborder Pharyngobdellae			
Family Erpobdellidae			
Family Trematobdellidae			
Family Semiscolecidae			

TABLE XXIV. LEECHES DETRIMENTAL TO OR PREDATORS OF MAN AND DOMESTIC ANIMALS

Organism	Habitats	References
	Aquatic Leeches	
Dinobdella ferox	India, Burma, and Ceylon	38
Haementeria officinalis	Mexico and South America	38
Haemophis cavillina	Italy	64
Hirudo medicinalis	Europe; South and East Asia; Africa; North America; South Wales; Islay, Scotland; Kalinin, U. S. S. R; and Hanover, Germany	38, 67, 68 69
Hirudo sanguisuga	British Isles	5
Hirundinaria manillensia	Malaya, Borneo, and Phillippines	10, 14, 38, 55, 64
Limnatis africana	Congo	64
Limnatis granulosa	India	64
Limnatis japonis	Japan	64
Limnatis maculosa	Singapore	64
Limnatis mysomelas	French West Africa (Senegal)	64
Limnatis nilotica	Southern Europe (Portugal, Italy, Spain, France, Greece, and Bulgaria), Northern Africa (Egypt, Tunis, Ethiopia, Algiers, and Morocco), the Azores, Canary Islands, Western Asia (Turkey, Syria, Armenia, Israel, Iran, Baluchistan, Afghanistan, and India), U. S. S. R., and Malaya	
Macrobdella decora	North America	38
Macrobdella valdiviana	South America	5
Placobdella Blanchard	Southern Europe, North America, Asia, and Africa	38
	Terrestrial Leeches	
Haemadipsa montana	India, Pakistan, and Burma	63, 64
Haemadipsa ornata	India, (Assam)	64
Haemadipsa picta	Malaya and Borneo	5, 11, 14, 18, 64
Haemadipsa sylvestris	Burma, Malaya, Borneo, Java, Sumatra, and India	13, 62, 63
Haemadipsa talagalla	Philippines	63
Haemadipsa zeylanica	Ceylon, India, Pakistan, Southwest Pacific Islands, Malaya, Borneo, and China	4, 5, 10, 11, 12, 14, 16, 17, 18, 38, 63, 64
Hirudinea	Chiapas, Mexico	70

While Table XXIV describes the general habitat of aquatic and terrestrial leeches, Table XXV gives the specific location of leeches identified in this study. Figure I graphically depicts the information provided in Table XXV.

IDENTIFICATION OF GAPS IN DEVELOPMENT OF EFFECTIVE LEECH REPELLENTS

Gaps have been identified in the past and present efforts in developing effective leech repellents. These are summarized as follows and are discussed below:

(1) Lack of knowledge of educational or motivational techniques which are effective in convincing indigenous people, colonials, and imported military and civilian personnel, to utilize leech repellents.

(2) Lack of noncontradictory basic knowledge of leech morphology and taxonomy from field studies.

(3) Lack of acceptable laboratory- or field-testing techniques for use in determining the effectiveness of leech repellents.

(4) Lack of current or continuing program to study terrestrial- and aquatic-leech repellents*.

While indigenous persons accept the leech as part of the environment, personnel imported into such areas are physiologically and psychologically affected adversely by the presence of leeches. Factors such as social mores, native culture, education, economic level, and repellent availability enter into the acceptance and use of leech repellents. Insect repellents M-1960, dimethyl phthlate, and diethyl toluamide, which are also effective as leech repellents, are not widely used because of their cosmetic unacceptability, unavailability, and lack of persistence. Nevertheless, they provide a degree of repellency which justifies their use by persons who consider leeches more intolerable than the repellent itself.

A further consideration related to leech repellents is that in military campaigns, troops can not often avoid leech-infested places. It is frequently necessary for troops to traverse and even remain in such territory according to the varying fortunes of the campaign. While the total loss of blood from bites may not be large, the blood nevertheless soils the uniforms at a time when laundry facilities are unavailable; hence, a psychological consideration becomes pertinent.

While several detailed morphological studies have been reported[38,60,64,65,72], the investigations have been concentrated on the Hirudo medicinalis or nonmammalian parasitic species. Traub and Newson[8] both indicated a need for such information on

*The U. S. Department of Agriculture, Gainesville, Florida, program on aquatic-leech repellents, if expanded, could fill this gap. However, as it is presently envisioned, it will involve only the study of aquatic leeches and "off the shelf" repellents, and is a short-term program.

TABLE XXV. SPECIFIC LOCATION OF LEECHES IDENTIFIED IN THIS STUDY

Organism	Occurrence	Map Location Code*	References
Hirundinaria manillensis	Borneo (Padas River Delta)	A	14
Haemadipsa picta	Borneo (Ranau)	B	14
Haemadipsa	Vietnam (Nong-Son near Tourane)	C	16
Haemadipsa zeylanica	Borneo (Mt. Kinabalu)	D	14, 17
	(Ranau)	E	14, 17
	Borneo, Sarawak (Kapit District) District)	F	18
	Ceylon (Ingiriya, 30 miles from Colombo	G	4
	(Diyatalawa, 120 miles from Colombo)	H	5
	(Ratnapura)	I	4
	Andaman Islands	J	5
	Burmese-Indian Border	K	4
Haemadipsa limatac	Philippines (Pampanga)	L	6
Haemadipsa sylvestris	India (Assam)	M	13
Limnatis	India (United Provinces, Lansdowne)	N	71

*Refer to Figure I.

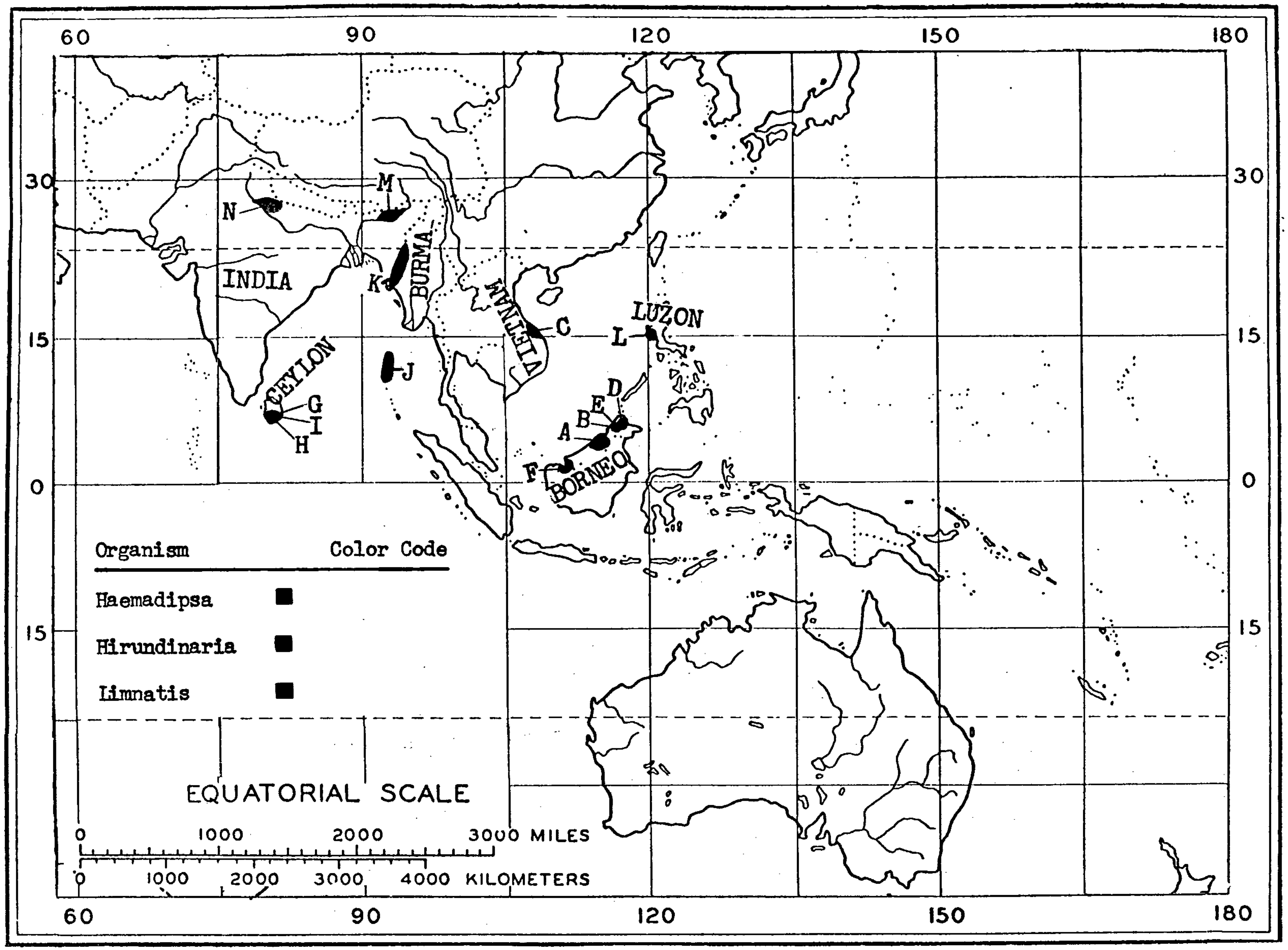

FIGURE 1. OCCURRENCES OF LEECHES REPORTED IN THIS STUDY

(The Letter Designation Specifies the Location Given in Table XXV)

tropical species; however, the qualified and dedicated scientists needed to do such work have not become available. As pointed out previously and indicated in Table XXIII, the taxonomy of leeches leaves much to be desired. The possibility of several of the organisms listed in Table XXIV being synonymous can be resolved only through studies on live and recently killed organisms. From Table XXIV it is evident that there is a lack of information on specific habitats of both terrestrial and aquatic leeches. Such information would be highly useful as a basis for providing military and other imported personnel with a leech repellent should it be necessary and effective in such areas.

The evaluation of potential leech-repellent compounds must be considered from the standpoint of a standard testing technique(s). There is a genuine need for the laboratory methods listed in Table XX to be examined closely and compared with field tests for validity and reliability, before any extensive long-term repellent screening program could be reasonably initiated. The possibility of developing standards or controls such as (1) the number of leech bites sustained in the field by persons wearing M-1960-impregnated standard U. S. Army issue uniforms, (2) the number of leech bites on persons using skin applications of diethyl toluamide in the field, and then (3) a comparison of the Items (1) and (2) data with similar data obtained in the laboratory merits considerable investigation. The laboratory results obtained by using freshly killed animals, live animals, human volunteers, impregnated cloth strips, or impregnated filter paper in the evaluation of leech repellency should be examined for their comparability with actual field repellency test data obtained on humans. Further, the effects of temperature, humidity, rubbing, wetting, perspiration, and illumination should be considered carefully in developing the laboratory testing technique. Methods of counting bites or leeches on clothing may be critical especially in areas where incisions from thorny undergrowth may resemble the results of leech bites to the extent that the count of bites may be beclouded; discriminating methods for making such counts should be developed.

Both terrestrial and aquatic leeches are a serious problem in tropical areas of the world. However, until malaria, anemia, malnutrition, and typhus, along with other tropical diseases, are eliminated, the effects of leech attack are of secondary concern. While the question has been raised as to whether leeches are vectors of disease, no field study to date has indicated that leeches carry diseases as is the case of the mite carrying scrub typhus. Boynton's[1] and Shope's[2] laboratory study of leeches as potential virus vectors suggests the need for more detailed laboratory and field testing. The information obtained from such an effort might provide a basis for leeches being assigned a higher priority of interest.

The repellents which have been investigated have certain objectionable features which limit their widespread utilization by personnel and natives in leech-infested areas. In the case of M-1960, the repellent has a distinctive odor, ruins plastic materials, and is persistent only through two washings of impregnated uniforms. Diethyl toluamide, while more acceptable cosmetically, is even more soluble in water than the M-1960, and so is less persistent.

The investigation by Stammers[4] was the only leech-repellent screening study which considered a number of potential compounds. The other contemporary studies which have been cited were of secondary concern to the investigators, who reported their observations of the leech-repellent tendencies of insect repellents. The most gratifying aspect of contemporary studies was the effort to conduct detailed experiments, with the

limited facilities available, by careful volunteer selection, use of control groups, considered methods of counting leech bites, and the study of available alternative repellent or protective measures.

Ointments tested to date have been only partially successful as leech repellents. Of those tested, dimethyl phthalate with white wax and arachis oil was the best, since it spreads well on skin and leather, and the wax and oil provide persistency by delaying the evaporation of the active material. The good initial protection offered by ointments soon breaks down, however, most likely because of the mechanical removal of the repellent and binder by water and mud.

The vapor tension of various compounds appears to be significantly conducive to improved repellency, and also to decreased adherence of leeches to clothes or to the skin. The addition of an emulsifier to the repellent compound to enhance the water solubility and thus facilitate uniform impregnation may have the adverse tendency to act as a reducer of repellency. A fatty type of emulsifier such as a distilled monoglyceride (available from Distillation Products Industries) might be used as a carrier for many of the repellents which have been suggested. These monoglycerides emulsify readily, and after the emulsion was impregnated into clothing, the water phase would evaporate and the repellent would be firmly fixed to the cloth. These materials have the added advantage of being completely nontoxic. A cosmetic type of lotion using stearic acid suspensions might also be employed as a repellent carrier. Furthermore, many silicones might productively be studied as carriers.

Differences in the effectiveness of various concentrations of potential repellent compounds have been indicated. For example, Buu-Hoi[16] reports that 200 grams of n-butylacetanilide in 11 liters of water (18 grams per liter) provided an effective clothing impregnant, while Traub[18] indicated that 37.5 grams of n-butylacetanilide in 3.785 liters (10 grams per liter) of water was ineffective as a clothing impregnant. Buu-Hoi did not report the specific organisms; he stated only that "terrestrial leeches of the Haemadipsa family", were the organisms studied. It would be of considerable interest to determine conclusively whether the difference in effectiveness as a repellent was attributable to the difference in concentration of the n-butylacetanilide, the specific species of leech involved, or the experimental method of evaluation used. In view of the pertinent uncertainties, the above-cited data may not be conclusive.

The effect of the mechanical barrier provided by clothing or in some cases by a repellent such as coconut oil cannot be underestimated. Uniforms properly worn provide excellent protection against the water leech (Hirundinaria manillensis) which because of its size, 15 to 20 centimeters long, cannot penetrate the cloth. On the other hand, the Limnatis nilotica, because of its ability to change its shape and diameter temporarily, may penetrate relatively small openings as do many terrestrial leeches. The young Limnatis nilotica is of major concern because of its search for the dark body openings and resulting difficulties to the human attacked. Uniforms are thus a critical problem in the tropics particularly since the currently used fatigue uniform is considered to be too heavy; and hence, portions are removed, and skin areas are exposed to leech attack. The uniform which is currently issued is an 8-1/2-ounce, fairly openly woven cotton fabric. A lightweight tropical uniform was used by Traub and Newson; however, the issuance of such a uniform has apparently been discontinued. A new tropical uniform has been developed that is made from 5-1/2-ounce cotton poplin, which is tightly woven thin material. The new uniform material was also developed for

the purpose of providing insect protection, which it does afford; hence, it will undoubtedly provide at least some mechanical protection from leeches. Uniform development programs should therefore be followed because of their applicability to the problem of leech repellency.

RECOMMENDATIONS

Since our nation has a considerable economic stake in the tropics, concern for the political developments in such nations, and an increasing dependency on such parts of the world for essential raw materials problems in tropical medicine and biology assume a greater importance than has been heretofore recognized. The leech-repellent problem, in view of this recognition, should be considered as an important research and development area. The immediate approach to the leech-repellent problem is to educate military and native personnel in the use of M-1960-repellent-impregnated uniforms and proper uniform wear. Because of the heat in tropical areas, the proper wearing of the uniform, which should be Standard Operating Procedure, is frequently disregarded. Also, as an additional repellency measure, diethyl toluamide should be applied directly to the skin, and while it is not as persistent as M-1960 impregnated into uniforms, nevertheless may afford considerable protection against terrestrial leeches.

The problem of replenishing the concentration of repellent in impregnated uniforms could be eliminated by adopting the tactics used by the British, i.e., by air lifting or somehow providing complete new sets of uniforms, footwear, and netting impregnated with repellent every 10 days to troops in combat zones along with food, ammunition, and other medical supplies.

On the basis of this study, it appears that a long-term repellent program should be initiated to run concurrently with the presently active short-term (18 months) U. S. Department of Agriculture study on "off the shelf" aquatic-leech repellents; such a program should be directed toward filling the gaps in our knowledge of both aquatic and terrestrial leeches, and toward developing an effective repellent for both aquatic and terrestrial organisms. The long-range program should include studies of: (1) leech feeding habits, (2) breeding, (3) taxonomy, (4) natural and synthetic chemical poisons as leech controls, (5) disease vectors, (6) repellent development and evaluation, (7) natural predators, (8) education of personnel and natives in the use of repellents, (9) repellent-impregnated articles which would be used by natives in Vietnam, Thailand, and other leech-inhabited areas, such as rope or canvas sandals, and (1) reported natural immunity among natives in Ceylon.

A long-range program to fill in the gaps in our knowledge of leeches and to develop improved leech repellents would most profitably be divided into phases. Since those who have reported repellent studies have performed such investigations as a secondary research effort, it will be necessary to develop and train personnel to conduct primary and decisive leech-repellent studies. A long-range program which might be set up to fill the important gaps in our knowledge of leeches would include the following:

(1) Phase I.

A field study should be conducted on Haemadipsa picta, Haemadipsa zeylanica, Haemadipsa sylvestris, Hirundinaria manillensis, and Limnatis nilotica to develop standard testing techniques. A research team composed of scientists with training in taxonomy, entomology, zoology, physiology, biochemistry, and psychology would perform a field study on repellents and leeches, and also collect specimens for use in developing comparative standard laboratory testing techniques; the development of the standard tests could be conducted in overseas medical research laboratories operated by the Military. The data obtained would be compared with the results of the current effort at the U. S. Department of Agriculture, Gainesville, Florida, on the laboratory testing of domestic aquatic-leech repellents. If it is possible to obtain in the laboratory results which are comparable to field data, the screening of the many potential repellent compounds could be performed with less effort and expense than is the case if field evaluation is necessary.

(2) Phase II.

Concurrent with the Phase I program would be a detailed study of leech morphology, chemical sensitivity of leeches, and the response of leeches to other stimuli. Continued laboratory testing of leech repellents would be performed and the most promising would be field tested.

(3) Phase III.

Concurrent with the first two phases would be the development of leech taxonomy and this effort would continue into a third phase. This final phase would be concerned with a coherent, well-founded taxonomy and with field testing of the effectiveness of leech repellents developed in Phases I and II, in various remote tropical regions.

Further, a research program on the development of techniques of educating natives is necessary, both relative to the use of leech repellents as well as in other health and technical training. How one proceeds to instill a desire in or to motivate people indigenous to the areas of interest is a research area in itself. The need for this kind of knowledge cannot be overlooked when dealing with underdeveloped nations.

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APPENDIX A

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Biological Abstracts Volumes 1 to 42, Number 5	January, 1926 to June 1, 1963
Chemical Abstracts Volumes 1 to 58, Number 12	1907 to June 10, 1963
Index Medicus Volumes 1 to 4, Number 6	January, 1960 to June, 1963
International Abstracts of Biological Sciences (British Abstracts of Medical Sciences) Volumes 1 to 29, Number 2	1954 to May, 1963
Current List of Medical Literature Volumes 1 to 36	January, 1941, to December, 1959
Excerpta Medica Medical Microbiology, Immunology, and Serology, Section IV Volumes 1 to 16	January, 1948 to April, 1963
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Abstracts of Japanese Medicine Volumes 1 and 2	October, 1961 to December, 1962
Abstracts of Soviet Medicine Basic Medical Sciences Volumes 1 to 4	 1957 to 1960
Clinical Medicine Volumes 1 to 4	 1957 to 1960

Tropical Diseases Bulletin Volumes 56 to 60	January, 1959 to May, 1963
Acta Medica Vietnamica Volumes 1 to 6	Issue by issue search, January, 1959 to May, 1963
Armed Forces Medical Library Catalog	1950 to 1954
National Library of Medicine Catalog	1955 to 1962
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APPENDIX B

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APPENDIX C

COST OF CHEMICALS USED IN
LEECH-REPELLENT FORMULATIONS

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COST OF CHEMICALS USED IN LEECH-REPELLENT FORMULATIONS

Chemical	Cost/Unit	Supplier
Aldrin	\$0.50-1.00/pound	Shell Chemical Co.
Arachis oil	\$.80/pint	Fisher Chemical Co.
Benzyl benzoate	\$5.25/kg	Eastman
n-butylacetanilide	\$4.35/100 g	Eastman
2-butyl-2-ethyl-1,3-propanediol	\$3.00/100 g	Aldrich Chemical Co., Inc.
Dibutyl phthalate	\$2.95/kg	Eastman
Dichlorodiphenyltrichlorethane (DDT)	\$0.25/pound	Montrose Chemical Allied Chemical Corp. Olin Mathieson Diamond Alkali Lebanon Chemical Co.
Dieldrin	\$1.00 to 2.00/pound	Shell Chemical Co.
Diethyl toluamide	\$2.00 to 3.00/pound	Hercules Powder Co. Montrose Chemical
Dimethyl carbate	\$6.40/kg	Eastman
Dimethyl phthalate	\$0.25/pound	Monsanto Chemical DuPont Allied Chemical Corp.
2-ethylhexanediol	\$7.45/kg	Union Carbide Eastman
Hydroxycitronellal	\$7.55/pound in 25-lb lots	Magnus, Mabee & Reynard Fritszche Bros.
Indalone (butyl mesityloxide oxalate)	\$0.50 to 1.00/pound	Niagara Chemical Div. FMC Corp.
Lindane	\$2.00 to 3.00/pound	Diamond Alkali Hooker Chemical Corp. Frontier Chemical

COST OF CHEMICALS USED IN LEECH-REPELLENT FORMULATIONS
(Continued)

Chemical	Cost/Unit	Supplier
Nicotine sulphate	\$0.50 to 1.00/pound	Black Leaf Products
n-propylacetanilide	~\$10.40/pound	Eastman
Santocel C	Less than \$1/pound	Monsanto Chemical Co.
Tween 80	\$2.00/pint	Atlas Powder
Undecylenic Acid	\$4.55/100 g	Eastman

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